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Devoted to the Advancement of the Biological Sciences
With Special Reference to the Factors of Evolution

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THE
AMERICAN NATURALIST

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THE EVOLUTION OF THE CELL[1]

BY THE LATE PROFESSOR E. A. MINCHIN, F.R.S.

WHEN addressing an audience of biologists it would be superfluous to insist upon the importance of the study of the cell and its activities. It is now recognized almost universally that the minute corpuscles known by the somewhat unsuitable term "cells" are the vital units of which the bodies of animals and plants are built up, and that all distinctive vital processes—metabolism, growth and reproduction, sexual phenomena and heredity—reduce themselves ultimately to activities taking place in, and carried on by, the individual cells which build up the body as a whole. Each cell must be regarded as a living, individual organism which, however much it may be specialized for some particular function or form of vital activity, is capable of maintaining its life and existence in a suitable environment by carrying on all the necessary processes of metabolism which are the essential and distinctive characteristics of living beings. In the case of cells composing the complex body of the higher animals and plants the cells are mutually interdependent, and, with the exception of the mature germ-cells, can not maintain their existence apart from their fellows; that is to say, the only natural[2] environment suitable for their con-

[1] Address by the President to the Zoölogical Section of the British Association for the Advancement of Science. Manchester, 1915.

[2] It is not necessary to do more than refer here to the investigations that have been carried on in recent years with regard to the viability and multiplication of tissue-cells removed from the body in artificial culture-media.

tinued existence is the complex body or cell-commonwealth of which they form an integral part. But in the simplest forms of life the whole body of the living individual may reach no higher degree of complexity than the single cell, which is then seen as an organism physiologically complete in every respect, living a free and independent life in Nature and competing with other organisms of all kinds, simple or complex, in the universal struggle for existence amongst living beings. This statement of the "cell-theory" is that with which, I believe, the majority of modern biologists would agree; not without, however, some dissentients, amongst whom I personally am not to be numbered.[3]

The fundamental importance of the cell as a complete living organism, whether maintaining itself singly and independently or in union with other similar but individually specialized units, has made it the object of intensive and concentrated study, not only by those who group themselves according to their special points of view as zoologists, botanists, physiologists, etc., but also by a class of investigators who take the cell itself as the subject of a branch of biological investigation termed cytology, which deals with cells in a general manner independently of their provenance, whether animal or vegetable. Some knowledge of the cell and its activities is necessary at the present time for every one concerned with the study of living things, whether that study is pursued for its own sake and with disinterested objects, or with the intention of applying scientific principles to practical aims, as in medicine or agriculture. One might have expected, therefore, that at least some elementary understanding of the nature and significance of the cell, and the importance of cellular activities in the study of life and living things, would have formed at the present time an indispensable part of the stock of knowledge acquired by all intelligent persons who are ranked as "educated" in popular esti-

These experiments afford strong support to the view that the cell is to be regarded primarily as an independent living organism.

[3] See Appendix A.

mation. Unfortunately this is so far from being the case that it is practically impossible, in this country at least, to find any one amongst the educated classes to whom the words "cell" and "cytology" convey any meaning at all, except amongst those who have interested themselves specially in some branch of biology. Consequently, any discussion concerning the cell, although it may deal with the most elementary processes of life and the fundamental activities and peculiarities of living beings, ranks in popular estimation as dealing with some abstruse and recondite subject quite remote from ordinary life and of interest only to biological specialists. It must, however, be pointed out that the general state of ignorance concerning these matters is doubtless in great part due to the fact that an objective acquaintance with cells can not be obtained without the use of expensive and delicate optical instruments.

I propose in this address to deal with an aspect of cytology which appears to me not to have received as yet the attention which it deserves, namely, the evolution of the cell itself and of its complex organization as revealed by the investigation of cytologists. Up to the present time the labors of professed cytologists have been directed almost entirely towards the study of the cell in its most perfect form as it occurs in the Metazoa and the higher plants. Many cytologists appear indeed to regard the cell, as they know it in the Metazoa and Metaphyta, as the beginning of all things, the primordial unit in the evolution of living beings.[4] For my part I would as soon postulate the special creation of man as believe that the Metazoan cell, with its elaborate organization and its extraordinarily perfected method of nuclear division by karyokinesis, represents the starting-point of the evolution

[4] For example, my friend Dr. C. E. Walker, in an article in *Science Progress* (Vol. VII, p. 639), after stating that "The unit of living matter, so far as we know, is the cell," proceeds to deal with "that form in which it is found in the multicellular and the majority of unicellular organisms, both animal and vegetable" and then describes the typical cell of the cytologist, with nucleus, cytoplasm, centrosome, chrondriosomes, and reproduction with fully developed karyokinesis.

of life. So long, however, as the attention of cytologists is confined to the study of the cells building up the bodies of the higher animals and plants, they are not brought face to face with the stages of evolution of the cell, but are confronted only with the cell as a finished and perfected product of evolution, that is to say, with cells which, although they may show infinite variation in subordinate points of structure and activity, are nevertheless so fundamentally of one type that their plan of structure and mode of reproduction by division can be described in general terms once and for all in the first chapter of a biological text-book or in the opening lecture of a course of elementary biology.

One of the most striking features of the general trend of biological investigation during the last two decades has been the attention paid to the Protista, that vast assemblage of living beings invisible, with few exceptions, to the unassisted human vision and in some cases minute beyond the range of the most powerful microscopes of to-day. The study of the Protista has received in recent years a great stimulus from the discovery of the importance of some of the parasitic forms as invaders of the bodies of men and animals and causers of diseases often of a deadly nature; it has, however, yielded at the same time results of the utmost importance for general scientific knowledge and theory. The morphological characteristic of the Protista, speaking generally, is that the body of the individual does not attain to a higher degree of organization than that of the single cell. The exploitation, if I may use the term, of the Protista, though still in its initial stages, has already shown that it is amongst these organisms that we have to seek for the forms which indicate the evolution of the cell, both those lines of descent which lead on to the cell as seen in the Metazoa and Metaphyta, as well as other lines leading in directions altogether divergent from the typical cell of the text-book. We find in the Protista every possible condition of structural differentiation and elaboration, from cells

as highly organized as those of Metazoa or even, in some cases, much more so, back to types of structure to which the term cell can only be applied by stretching its meaning to the breaking-point. Already one generalization of cytologists has been torpedoed by the study of the Protista. The dictum "Omnis nucleus e nucleo" is perfectly valid as long as it is restricted to the cells of Metazoa and Metaphyta, to the material, that is to say, to which the professed cytologist usually confines his observations.[5] But in the Protista it is now well established that nuclei can arise *de novo*, not from preexisting nuclei but from the extranuclear chromatin for which Hertwig first coined the term "chromidia."

It is clear, therefore, that the results already gained from the study of the Protista have brought about a new situation which must be faced frankly and boldly. It is impossible any longer to regard the cell as seen in the Metazoa and as defined in the text-books as the starting-point of organic evolution. It must be recognized that this type of cell has a long history of evolution behind it, which must be traced out, so far as the data permit. The construction of phylogenies and evolutionary series is of course purely speculative, since these theories relate to events which have taken place in a remote past, and which can only be inferred dimly and vaguely from such fragments of wreckage as are to be found stranded on the sands of the time in which we live. Many important stages of evolution may be totally submerged and no longer available for study and consideration. The extent to which such speculations will carry conviction to a reasonable mind will depend entirely on the stores of

[5] Vejdovský ("Zum Problem der Vererbungsträger," Prag, 1911–1912, p. 120) has already maintained, for the cells of Metazoa, that Fleming's aphorism "Omnis nucleus e nucleo" should be changed to "Omnis nucleus e chromosomatis" [*sic*], on the ground that the nucleus, as such, is not an original cell-component "but is produced secondarily from the chromosomes of the mother-cell." If this is true, there is but little difference in detail, and none in principle, between the formation of "secondary" nuclei from chromidia and the reconstruction of a daughter-nucleus from chromosomes in the most perfected form of karyokinesis.

data that can be collected and which must be the last appeal for the cogency of all arguments and judgments. The study of the Protista is as yet in its infancy; groups have been recognized and have received ponderous designations, although their very existence is yet in doubt, as in the case of the so-called Chlamydozoa; and our knowledge of the affinities and mutual relationships of the groups is still very imperfect. All attempts, therefore, to trace the evolution of the Protista must be considered as purely tentative at present. If I venture upon any such attempt, it is to be regarded as indicating a firm belief on my part that the evolution of the cell has taken place amongst the Protista, and that its stages can be traced there, rather than as a dogmatic statement that the evolution has taken place in just the manner which seems to me most probable. When we reflect on the irreconcilable differences of opinion amongst zoologists with regard to the origin and ancestry of vertebrates, for example, we may well be cautious in accepting pedigrees in Protista.

Before, however, I can proceed to deal with my main subject, it is absolutely necessary that I should define clearly the sense in which I propose to use certain terms, more especially the words "cell," "nucleus," "chromatin," "protoplasm" and "cytoplasm." Unless I do so my position is certain to be misunderstood, as, indeed, it has been already by some of my critics.

The term cell was applied originally by botanists to the single chambers or units of the honeycombed structure seen in the tissues of plants. The application of the term to such structures is perfectly natural and intelligible, since each such cell in its typical form is actually a closed space limited by firm walls, and containing a relatively large quantity of fluid cell-sap and a small quantity of the slimy protoplasmic substance. When these structures were first discovered, the limiting membrane or wall of the cell was regarded as essential, and less importance was attached to its contents. With increased knowledge,

however, and especially when animal tissues came to be studied, it became apparent that the cell-wall, like the fluid cell-sap, was a secondary product, and that the essential and primary part of the cell was the viscid protoplasmic substance, in which a peculiar body, the "nucleus," or kernel, was found to be universally present. Consequently the application and meaning of the term cell had to undergo an entire change, and it was defined as a small mass or corpuscle of the living substance, protoplasm, containing at least one nucleus. To these essential constituents other structures, such as a limiting membrane or cell-wall, and internal spaces—vacuoles—filled with watery fluid, might be added as products of the secretory or formative activity of the living substance; but such structures were no longer regarded as essential to the definition of the cell, since in many cases they are not present. It is to be regretted in some respects that with this changed point of view the term "cell," used originally under a misapprehension, was not replaced by some other term of which the ordinary significance would have been more applicable to the body denoted by it.[6]

The chief point that I wish to establish, however, is that the term cell was applied originally to the protoplasmic corpuscles building up the bodies of the Metazoa and Metaphyta, each such corpuscle consisting of a minute individualized mass of the living substance and containing a nucleus. Hence a complete cell is made up of two principal parts or regions, the nucleus and the remainder of the protoplasmic body, termed the cytoplasm. By some authors the term protoplasm is restricted to the cytoplasmic portion of the cell, and protoplasm is then contrasted with nucleus; but it is more convenient to consider the whole cell as composed of protoplasm divided into two regions, nucleus and cytoplasm.

We come now to the consideration of the body termed

[6] "Nothing could be less appropriate than to call such a body a 'cell'; yet the word has become so firmly established that every effort to replace it by a better has failed, and it probably must be accepted as part of the established nomenclature of science."—E. B. Wilson, "The Cell," p. 19.

the nucleus, which undoubtedly possesses an importance in the life and functions of the cell far greater than would be inferred from the name given to it. A nucleus, as seen in its typical form, has a limiting membrane enclosing a framework composed of a substance termed "linin." The framework has the form of a network, which is probably to be interpreted, primitively at least, as the optical expression of an alveolar structure similar to that seen also in the cytoplasm, but of coarser texture, and the apparent "threads" of the linin-framework may then be the optical sections of the partitions between neighboring alveoli. Such an interpretation does not exclude the possibility of the formation of real threads or fibers in the framework in certain cases or during particular periods of nuclear activity; just as fibrous structures may arise in the alveolar cytoplasm also. The cavities of the framework contain a watery fluid or nuclear sap, probably of the same nature as the fluid enchylema or cell-sap contained in the alveolar framework of the cytoplasm. At the nodes of the alveolar framework are lodged grains or masses of *chromatin,* a substance which must engage our most particular attention, since it is the essential constituent of the nucleus, universally present in all nuclei, whether of the simplest or of the most complex types. In addition to the chromatin-grains, which are distributed in various ways over the linin-framework, there are to be found usually one or more masses termed nucleoli, composed of a material which differs from chromatin in its reactions and has been termed plastin.

In the foregoing paragraph I have described in general terms the typical nucleus of the text-books, as found commonly in the cells that build up the bodies of ordinary animals and plants. The minutiæ of the details of structure and arrangement of the constituent parts may vary infinitely, but the type remains fairly constant. When we come, however, to the nuclei of the Protista, such pronounced modifications and variations of the type are met with that a description in general terms is no longer pos-

sible. I shall deal with some of these types later in my attempts to reconstruct the evolution and phylogeny of the cell. I will draw attention now only to a few salient points. In the Protist cell the chromatin is not necessarily confined to the nucleus, but may occur also as extranuclear grains and fragments termed chromidia, scattered through the protoplasmic body; and the chromatin may be found only in the chromidial condition, a definite nucleus being temporarily or permanently absent. Further, when a true nucleus is present in the Protist body, it seldom contains a nucleolus of the same type as that seen in the nuclei of tissue-cells, that is to say, a mass of pure plastin, but in its place is found usually a conspicuous body which shows reactions agreeing more or less closely with those of chromatin and which consists of a plastin-basis more or less densely impregnated with chromatin. Such a body is termed a karyosome (or chromatin-nucleolus) to distinguish it from the true nucleoli (plastin-nucleoli) characteristic of tissue-cells. According as the plastin or the chromatin predominates in the composition of a karyosome, its reactions may resemble more nearly those of a true nucleolus in the one case, or those of chromatin in the other. The so-called karyosomatic type of nucleus is very common in the Protista, but by no means of invariable occurrence; in many cases the nucleus consists of a clump of small grains of chromatin, with no distinct karyosome, or with a karyosome which consists mainly of plastin. Thus two extreme types of nuclear structure can be distinguished and may be termed provisionally the karyosomatic type and the granular type, ignoring for the sake of convenience in nomenclature the types of structure transitional between the two; as, for example, types in which a distinct karyosome is seen together with more or fewer peripherally arranged grains of chromatin.

In either the karyosomatic or the granular type of Protist nucleus we may find great simplification of the complex type of nuclear structure seen in the tissue-cells

of animals and plants. Thus in the first place a distinct nuclear membrane may be entirely absent and the chromatin-elements, whether occurring in the form of a compact karyosome or of a clump of grains, are lodged simply in a vacuole in the cytoplasm, that is to say in a cavity containing a watery fluid of nuclear sap in which the mass or masses of chromatin are suspended. It is a moot point, to which I shall return again, whether in nuclei of this simple type the linin-framework may sometimes be absent altogether, or whether it is invariably present in at least a rudimentary form, appearing as delicate threads (in optical section) extending from the chromatin-masses to the limiting wall of the nuclear vacuole, or between the grains of chromatin themselves. When such a framework can be detected, the nucleus acquires the appearance, in preserved preparations at least, of possessing a definite structure and is often termed a resting nucleus; many observations have shown, however, that the nucleus during life is undergoing continual internal movements and re-arrangements of its parts and is by no means at rest. The linin-framework can not, therefore, be regarded in any way as a rigid skeleton, but must be interpreted as an alveolar framework similar to that of the general protoplasm and equally liable to movement, displacement and change.

From this survey, necessarily most brief and superficial, of the manner in which the nuclei of Protists may vary from the type of nucleus described in the text-books, it is at once evident that the essential part of the nucleus is the chromatin, and that the other structural constituents of the nucleus, namely, membrane, framework, and plastin or nucleolar bodies, are to be regarded as accessory components built up round, or added to, the primary nuclear material, the chromatin. Even with regard to the nuclei of Metazoa it is maintained by Vejdovsky that at each cell-generation the entire nucleus of the daughter-cell is produced from the chromosomes alone of the

mother cell.[7] The simplest body which can be recognized as a nucleus, distinct from the chromidia scattered without order or arrangement throughout the protoplasmic body, is a mass of chromatin or a clump of chromatin-grains supported on a framework and lodged in a special vacuole in the cytoplasm. The complexity seen in the most perfect type of nucleus takes origin by progressive elaborations of, and additions to, a structure of this simple and primitive type.

This brings me to a point which I wish to emphasize most strongly, namely, that the conception of a true cell-nucleus is essentially a structural conception. A nucleus is not merely an aggregation of chromatin; it is not simply a central core of some chemical substance or material differing in nature from the remainder of the protoplasm. As Dobell has well expressed it, a pound of chromatin would not make a nucleus. The concepts "nucleus" and "chromatin" differ as do those of "table" and "wood." Although chromatin is the one universal and necessary constituent entering into the composition of the cell-nucleus, a simple mass of chromatin is not a nucleus.[8] A true nucleus is a cell-organ, of greater or less structural complexity, which has been elaborated progressively in the course of the evolution of the cell;

[7] Walker, on the other hand, considers that "it seems quite possible that the chromatin is merely a secretion of the linin." (*Science Progress*, Vol. VII, p. 641.) I doubt whether there are many cytologists who would admit this possibility, and I think that very few protistologists would assent to any such notion, since in the nuclei of Protista the linin-framework is in many cases very little in evidence, if present at all.

[8] Professor Armstrong writes: "Every organism must possess some kind of nucleus, visible or invisible: some formative center round which the various templates assemble that are active in directing the growth of the organism." (*Science Progress*, Vol. VII, p. 328.) I need hardly point out that a chemical nucleus of this kind is not in the least what the biologist or cytologist means by the term cell-nucleus. The one is a subjective postulate necessary for the comprehension of the activities of any speck of living matter or any portion, however minute, of a living organism; the other is a concrete structure, known to us by actual observation, and as much an integral part of the true cell, considered as a definite type of organism, as a backbone or its morphological equivalent is essential to the definition of a true vertebrate.

it is as much an organ of the cell as the brain is an organ of the human body. As a definite cell-organ, it performs in the life and economy of the cell definite functions, which it is the province of the cytologist to observe and to study, and if possible to elucidate and explain. As an organ of the cell, however, it has no homologue or analogue in the body of the multicellular animals or plants; there is no organ of the human body, taken as a whole, similar or comparable to the nucleus of the cell. Consequently, in studying the functions of the nucleus the human cytologist finds himself in the same difficult position that an intelligent living being lacking the sense of sight would be when trying to discover the function of visual organs in other organisms possessing that sense. There is no organ of known and understood functions with which the cytologist can compare the cell-nucleus directly.

The foregoing brief consideration of the nucleus leads me now to discuss in more detail the nature and properties of the essential nuclear substance, the so-called chromatin. To define, or characterize adequately, this substance is a difficult task. The name chromatin is derived from the fact that this substance has a peculiar affinity for certain dyes or stains, so that when a cell is treated with the appropriate coloring reagents—with so-called nuclear stains—the chromatin in the nucleus stands out sharply, by reason of being colored in a different manner from the rest of the cell. In consequence, the statement is frequently made, in a loose manner and without reflection, that chromatin is recognized by its staining reactions, but in reality this is far from being true. When a preparation of an ordinary cell is made by the methods of technique commonly in use, the chromatin is recognized and identified by its position in a definite body with characteristic structure and relations to the cell as a whole, namely the nucleus, and this is equally true whether the chromatin has been stained or not. When

the cell has been stained with one of the dyes ordinarily in use for coloring the chromatin, there are often seen in the cytoplasm grains that are colored in exactly the same manner as the chromatin-grains lodged in the nucleus. Is an extranuclear grain which stains like chromatin to be identified, *ipso facto,* as chromatin? By no means; it may or it may not be chromatin. Simple inspection of a stained preparation is altogether inadequate to determine whether such a body is or is not chromatin. Any so-called chromatin-stain colors many bodies which may occur in a cell besides the chromatin, and it may be necessary to try a great many different stains before a combination is found which will differentiate a given cytoplasmic enclosure from a true chromatin-grain by its color-reactions. The so-called volutin-grains, for example, which are found commonly in the cytoplasm of many Protists, are identified by the fact that they have a stronger affinity for "chromatin-stains" than chromatin itself.

When, moreover, chromatin is compared with regard to its staining-reactions, both in different organisms, and in the same organism at different times, it is found to react very differently to one and the same stain. A striking example of this capriciousness is seen when a preserved film is made of the blood of some vertebrate which has nucleated blood-corpuscles, such as a bird or fish, and which contains also parasitic trypanosomes. It is easy to stain the nuclei of the blood-corpuscles with various stains, as, for example, carmine-stains such as picro-carmine or alum-carmine, which will not color the nuclei of the trypanosomes in the slightest. Moreover, every cytologist knows that the "chromaticity" of the chromatin varies enormously in different phases of the nuclear cycle of generation; it is often difficult to stain the chromatin in the "resting" nucleus, but the first sign of impending nuclear division is a marked increase in the staining powers of the chromatin. There is no dye known which can be relied upon to stain chromatin always, or wherever

it occurs. Methyl-green has been claimed to be the most reliable and certain of nuclear stains, but R. Hertwig, in his classical researches upon *Actinosphærium*, showed that it sometimes fails to stain chromatin. It is perfectly conceivable that there might be varieties of chromatin which could not be stained by any dye whatsoever.

I have felt bound to insist strongly upon the inadequacy of staining-methods for the detection and identification of chromatin, well known though these facts are to every cytologist, because here also I note a tendency amongst biological chemists to regard staining-properties as the sole criterion of chromatin. In reality such properties are of entirely secondary importance. To use the terminology of formal logic, staining-properties are an "accident," though it may be an "inseparable accident," of chromatin, not a "difference" which can be used to frame a logical definition, *per genus et differentias*, of this substance. If chromatin were nothing more than "stainable substance," as Professor Armstrong terms it,[9] some of the most important results of cytological investigation would be deprived of all real significance and reduced to the merest futilities.

What then is the true criterion of the chromatin-substance of living organisms? From the chemical point of view the essential substance of the cell-nucleus would appear to be characterized by a complexity of molecular structure far exceeding that of any other proteins, as well as by certain definite peculiarities. Especially characteristic of chromatin is its richness in phosphorus-compounds, and it stands apart also from other cell-elements in its solvent reactions, for example, resistance to peptic digestion. E. B. Wilson, in his well-known treatise, has emphasized the "cardinal fact . . . that there is a definite and constant contrast between nucleus and cytoplasm." The outstanding feature of the nucleus is the constant presence in abundance of nuclein and nucleoproteins. Nuclein, which is probably identical with chromatin, is a

[9] *Science Progress*, Vol. VII, p. 327.

complex albuminoid substance rich in phosphorus. It is the phosphorus-content of chromatin that is its most characteristic chemical peculiarity as contrasted with the cytoplasm. How far these features are common, however, to all samples of chromatin in all types of living organisms universally, can not, I think, be stated definitely at present; at any rate, it is not feasible for a cytologist of these days to identify a granule in a living organism or cell as chromatin solely by its chemical reactions, although it is quite possible that at some future time purely chemical tests will be decisive upon this point—a consummation devoutly to be wished.

The only criterion of chromatin that is convincing to the present-day biologist is the test of its behavior, that is to say, its relations to the life, activity and development of the organism. I may best express my meaning by objective examples. If I make a preparation of *Arcella vulgaris* by suitable methods, I see the two conspicuous nuclei and also a ring of granules lying in the cytoplasm, stained in the same manner as the chromatin of the nuclei. Are these extranuclear granules to be regarded also as chromatin? Yes, most decidedly, because many laborious and detailed investigations have shown that from this ring of granules in *Arcella* nuclei can arise, usually termed "secondary" nuclei for no other reason than that they arise *de novo* from the extranuclear chromatin and quite independently of the "primary" nuclei. The secondary nuclei are, however, true nuclei in every respect, as shown by their structure, behavior and relations to the life-history of the organism; they may fuse as nuclei of gametes (pronuclei) in the sexual act and they become, with or without such fusion, the primary nuclei of future generations of *Arcella;* they then divide by karyokinesis when the organism reproduces itself in the ordinary way by fission, and are replaced in their turn by new secondary nuclei at certain crises in the life-history. In view of these facts it can be asserted without hesitation that the ring of staining granules in *Arcella* is composed of, or at

least contains, true chromatin-grains, extranuclear chromatin for which R. Hertwig's term chromidia is now used universally. It is interesting to note that until the life-history of *Arcella* was studied in recent times the conspicuous ring of chromidia was generally overlooked and is not shown in some of the older pictures of the organism.

If, on the other hand, I make a preparation of some unidentified amœba occurring casually in pond-water or in an infusion, and find in its cytoplasm certain grains staining in same manner as the chromatin of the nucleus, it is quite impossible, without a knowledge of the life-history of the organism, to assert definitely that the grains in question are or are not true chromidia. They might equally well turn out to be volutin or any other substance that has an affinity for the particular chromatin-stains used in making the preparation.

The fact that at the present time the only decisive criterion of what is or is not chromatin is supplied only by its behavior in the life-history and its relation to the organism, makes it much easier to identify the chromatin in some cases than in others. In those Protista or cells which contain, during the whole or a part of the life-history, one or more true nuclei, recognizable as such unmistakably by their structure and their characteristic relations to the reproductive and sexual phenomena of the organism, the chromatin can be identified with certainty. If chromidia occur in the cell-body in addition to true nuclei or even if the nuclei are temporarily absent during certain crises of the life-history and the chromatin occurs then only in the form of chromidia, there is still no difficulty in identifying the scattered chromatin-grains by the fact that they contribute, soon or later, to the formation of nuclei.

On the other hand, in the simplest Protist organisms which do not contain definite, compact nuclei recognizable by their structure and behavior, the identification of the chromatin may become correspondingly difficult. In the absence of definite chemical criteria the term chromatin

acquires then a greater or less degree of vagueness and uncertainty of application, and it is not easy to avoid a tendency to a *petitio principii* in attempting to define or identify it. To a large extent we are thrown back upon the staining-reactions, which I have already shown to be very unreliable, backed up by analogies with those forms which possess definite nuclei. Since in the cells of all animals and plants, and in all Protista which possess a true nucleus, the chromatin is the one constituent which is invariably present, as I shall point out in more detail subsequently, there is at least a strong presumption, though not of course amounting to absolute proof, that it is present, or at least is represented by some similar and genetically homologous constituents, in the forms of simpler structure also. If then in Protista of primitive type we find certain grains which exhibit the characteristic staining-reactions of chromatin to be constantly present in the organism, grains which grow and divide as a preliminary to the organism multiplying by fission and which are partitioned amongst the daughter-organisms during the process of fission, so that each daughter-individual reproduces the structure of the parent-form from which it arose; then there is very strong *prima facie* evidence, to say the least, for regarding such grains as homologous with the chromatin-grains of ordinary cells.

Having now defined or explained, as well as I am able, the terms of which I am about to make use, I return to my main theme, the cell and its evolution. To summarize the points already discussed, a typical cell is a mass of protoplasm differentiated into two principal parts or regions, the cytoplasm and the nucleus, or, it may be, two or more nuclei. The cytoplasm may or may not contain chromatin-grains in addition to other enclosures, and may possess cell-organs of various kinds. The nucleus, highly variable in minute structure, possesses one invariable constituent, the chromatin-material in the form of grains and masses of various sizes.

The cell, therefore, in its complete and typical form, is

an organism of very considerable complexity of structure and multiplicity of parts. The truth of this proposition is sufficiently obvious even from simple inspection of the structural details revealed by the microscope in cells in the so-called "resting condition," but still more so from a study of their activities and functions. The vital processes exhibited by the cell indicate a complexity of organization and a minuteness in the details of its mechanism which transcend our comprehension and baffle the human imagination, to the same extent as do the immensities of the stellar universe. If such language seems hyperbolic, it is but necessary to reflect on some of the established discoveries of cytology, such as the extraordinary degree of complication attained in the process of division of the nucleus by karyokinesis, or the bewildering series of events that take place in the nuclei of germ-cells in the processes of maturation and fertilization. Such examples of cell-activity give us, as it were, a glimpse into the workshop of life and teach us that the subtlety and intricacy of the cell-microcosm can scarcely be exaggerated.

On the assumption that an organism so complex and potent was not created suddenly, perfect and complete as it stands, but arose, like all other organisms, by progressive evolution and elaboration of some simpler form and type of structure, it is legitimate to inquire which of the various parts of the cell are the older and more primitive and which are more recent acquisitions in the course of evolution. But it must be clearly pointed out, to start with, that the problem posed in such an inquiry is perfectly distinct from, and independent of, another point which has often been discussed at length, namely, the question whether any parts of the cell, and if so which parts, are to be regarded as "living" or "active" in distinction to other parts which are to be regarded as "not-living" or "passive." This discussion, in my opinion, is a perfectly futile one, of which I intend to steer clear.

We may agree that in any given cell or living organism,

simple or complex in structure, all the parts are equally "living" and equally indispensable for the maintenance of life, or at least for the continuance of the vital functions in the normal, specific manner, without losing the right to inquire which of those parts are the phylogenetically older. A simple analogy will serve to point my meaning. A man could not continue to live for long if deprived either of his brain, his digestive tract, his lungs, his heart, or his kidneys, and each of these organs is both "living" in itself and at the same time an integral part of the entire organization of the human body; yet no one would think of forbidding comparative anatomists to discuss, from the data at their command, which of these organs appeared earlier, and which later, in the evolution of the phylum Vertebrata. Moreover, speculative though such discussions must necessarily be, there is no one possessing even a first-year student's knowledge of the facts who would controvert the statement that the digestive tract of man is phylogenetically older than the lung. Speculative conclusions are not always those that carry the least conviction.

The evolution of the cell may be discussed as a morphological problem of the same order as that of the phylogeny of any other class or phylum of living beings, and by the same methods of inquiry. In the first place there is the comparative method, whereby different types of cell-structure can be compared with one another and with organisms in which the cell-structure is imperfectly developed, in order to determine what parts are invariable and essential and what are sporadic in occurrence and of secondary importance, and if possible to arrange the various structural types in one or more evolutionary series. Secondly there is the developmental or ontogenetic method, the study of the mode and sequence of the formation of the parts of the cell as they come into existence during the life-history of the organism. Both these methods, which are founded mainly on observation, require to be checked and controlled by the experimental

methods of investigating both the functions and behavior of the organism and of its parts.

So long as cytologists limit their studies to the cells building up the tissues of the higher animals and plants, the comparative method has a correspondingly limited scope, and that of the ontogenetic method is even more restricted. Both methods receive at once, however, an enormously extended range when the Protista are taken into consideration. Then, moreover, we see the dawning possibility of another method of investigation, that, namely, of the chemical evolution of the organisms. Already some of the simpler Protista, the Bacteria, are characterized and classified largely by their chemical activities; but in more complex organisms, in those which have attained complete cell-structure, such as Protozoa, the data of chemistry do not as yet supply the evolutionist with a helpful method of investigation.

The problem of cell-evolution may be attacked by the help of the methods outlined in the foregoing remarks, beginning with the consideration of the primary structural differentiation of the typical cell, the distinction of nucleus, or rather chromatin, and cytoplasm. Since all cells known to us exhibit this differentiation, we have three possibilities as regards the manner in which it has come about, which may be summarized briefly as follows: either the cytoplasmic and chromatinic constituents of the cell have arisen as differentiations of some primitive substance, which was neither the one nor the other; or one of these two substances is a derivative of the other, in the course of evolution, either cytoplasm of chromatin, or chromatin of cytoplasm.

The idea of a primitive, undifferentiated protoplasmic substance was first put forward by Haeckel, who employed for it the term "plasson" invented by Van Beneden[10] to denote "la substance constitutive du corps des Monères et des cytodes . . . le substance formative par

[10] *Bull. de l'Acad. Roy. de Belgique*, Second Series, Vol. XXXI (1871), p. 346.

excellence." The simplest elementary organisms were not cells, but cytodes, "living independent beings which consist entirely of a particle of plasson; their quite homogeneous or uniform body consists of an albuminous substance which is not yet differentiated into karyoplasm and cytoplasm, but possesses the properties of both combined."[11] It is emphasized[12] that a sharp distinction must be drawn between protoplasm and plasson, the latter being a homogeneous albuminous formative substance ("Bildungsstoff") corresponding to the "Urschleim" of the older nature-philosophy.

Haeckel, as was usual with him, did not content himself with putting forward his ideas as abstract speculations, but sought to provide them with a concrete and objective foundation by professing to have discovered, and describing in detail, living and existing organisms which were stated to remain permanently in the condition of cytodes. In consequence, a purely speculative notion was permitted to masquerade for many years under the false appearance of an objective phenomenon of nature, until the error was discovered gradually and the phantom banished from the accepted and established data of biology. Organisms supposed to be of the nature of cytodes constituted Haeckel's systematic division, Monera, of which there were supposed to be two subdivisions, the Phytomonera and the Zoomonera. The Phytomonera were stated to have the plasson colored green and to live in a plant-like manner; the Zoomonera were colorless amœboid masses of plasson which nourished themselves in the animal manner. The Bacteria were also included by Haeckel in his Monera, apparently, or at all events ranked as cytodes.[13] Most importance, however, was attributed by Haeckel to the large amœboid forms of Monera, described as without nuclei or contractile vacuoles, but as representing simply structureless

[11] *Anthropogenie*, sixth edition, Leipzig, 1910, p. 119.

[12] *Ibid.*, p. 532.

[13] *Ibid.*, p. 119.

contractile masses of albumin ("Eiweiss"), perfectly homogeneous;[14] examples of these were announced to exist under the names "Protamœba" and "Protogenes," denoting forms of life which Haeckel claimed to have discovered, but which have never been found again by any other naturalist. These organisms, as described by Haeckel, were by no means such as the modern microscopist would call minute; on the contrary, they were relatively large, and some of the forms added to the Monera by Haeckel's contemporaries might even be termed gigantic, as, for example, the supposed organism *Bathybius,* discovered in the bottles of the *Challenger* Expedition, which was believed to cover large areas of the floor of the ocean with a layer of primordial protoplasm, but which proved finally to be a precipitation by alcohol of the gypsum in sea-water.

The theory of plasson and of the cytodes of Haeckel may be considered first from the purely speculative standpoint of the origin of the living substance, a problem with which I wish to become entangled here as little as possible, since it is my object to confine myself so far as possible to deductions and conclusions that may be drawn from known facts and concrete data of observation and experiment. If, however, we postulate a chemical evolution of protoplasm, and believe that every degree of complexity exists, or at least has existed, between the simplest inorganic compounds and the immensely complicated protein-molecules of which the living substance is composed, then no doubt chemical compounds may have existed which in some sense were intermediate in their properties between the two constituents, cytoplasm and chromatin, found in all known samples of the living substance of organisms. In this sense and on such a hypothesis, a substance of the nature of plasson may perhaps be recognized or postulated at some future time by the biochemist, but this is a subject which I am quite incompetent to discuss. To the modern biologist, who can deal

[14] See his "Prinzipien der generellen Morphologie," Berlin, 1906, p. 61.

only with living things as he knows them, Haeckel's plasson must rank as a pure figment of the imagination, altogether outside the range of practical and objective biology at the present time. All visible living things known and studied up to the present consist of protoplasm, that is to say, of an extremely heterogeneous substance of complex structure, and no living organism has been discovered as yet which consists of homogeneous structureless albuminous substance. Van Beneden, who is responsible for the word plasson, though not for the cytode-theory, was under the impression that he had observed a non-nucleated homogeneous cytode-stage in the development of the gregarine of the lobster, *Gregarina* (*Porospora*) *gigantea*. Without entering into a detailed criticism of Van Beneden's observations upon this form, it is sufficient to state that the development of gregarines is now well known in all its details, and that in all phases of their life-cycle these organisms show the complete cell-structure, and are composed of nucleus and cytoplasm. Moreover, all those organisms referred by Haeckel to the group Monera which have been recognized and examined by later investigators have been found to consist of ordinary cytoplasm containing nuclei or nuclear substance (chromatin). In the present state of biological knowledge, therefore, the Monera as defined by Haeckel must be rejected and struck out of the systematic roll as a non-existent and fictitious class of organisms.

Since no concrete foundation can be found for the view that cytoplasm and chromatin have a common origin in the evolution of living things, we are brought back to the view that one of them must have preceded the other in phylogeny. The theories of evolution put forward by Haeckel and his contemporaries, if we abolish from them the notion of plasson and substitute for it that of ordinary protoplasm, would seem to favor rather the view that the earliest forms of life were composed of a substance of the nature rather of cytoplasm, and that the nuclear substance or chromatin appeared later in evolu-

tion as a product or derivative of the cytoplasm. I have myself advocated a view diametrically opposite to this, and have urged that the chromatin-substance is to be regarded as the primitive constituent of the earliest forms of living organisms, the cytoplasmic substance being a later structural complication. On this theory the earliest form of living organism was something very minute, probably such as would be termed at the present day "ultra-microscopic." After I had urged this view in the discussion on the origin of life at the Dundee Meeting of the British Association in 1912 a poem appeared in *Punch*,[15] dividing biologists into "cytoplasmists" and "chromatinists." I must confess myself still a whole-hearted chromatinist. But before I consider this point I may refer briefly to some other speculations that have been put forward with regard to the nature of the earliest form of life. It is manifestly quite impossible that I should undertake here to review exhaustively all the theories and speculations with regard to the origin of life and the first stages in its evolution that have been put forward at different times. I propose to limit myself to the criticism of certain theories of modern times which, recognizing the fundamental antithesis between chromatin and cytoplasm, regard these two cell-constituents as representing types of organisms primitively distinct, and suggest the hypothesis that true cells have arisen in the beginning as a process of symbiosis between them. Boveri, whose merits as a cytologist need no proclamation by me, was the first I believe to put forward such a notion; he enunciated the view that the chromosomes were primitively independent elementary organisms which live symbiotically with protoplasm, and that the organism known as the cell arose from a symbiosis between two kinds of simple organisms, "Monera."[16]

A similar idea lies at the base of the remarkable and

[15] Vol. CXLIII, p. 245.

[16] *Fide* Vejdovsky, *l. c.* I have not had access to the work of Boveri, in which he is stated to have put forward these ideas.

ingenious speculations of Mereschkowsky,[17] who assumes a double origin for living beings from two sorts of protoplasm, supposed not only to differ fundamentally in kind but also to have had origins historically distinct. The first type of protoplasm he terms mycoplasm,[18] which is supposed to have come into existence during what he calls the third epoch[19] of the earth's history, at a time when the crust of the earth had cooled sufficiently for water to be condensed upon it, but when the temperature of the water was near boiling-point; consequently the waters of the globe were free from oxygen, while saturated with all kinds of mineral substances. The second type of protoplasm was amœboplasm, the first origin of which is believed to have taken place during a fourth terrestrial epoch when the waters covering the globe were cooled down below 50° C., and contained dissolved oxygen but fewer mineral substances. Corresponding with the differences of the epoch and the conditions under which they arose, Mereschkowsky's two types of protoplasm are distinguished by sharp differences in their nature and constitution.

Mycoplasm, of which typical examples are seen in bacteria, in the chromatin-grains of the nucleus and the chromatophores of plant-cells, is distinguished from amœboplasm, which is simply cytoplasm, by the following points. (1) Mycoplasm can live without oxygen, and did so in the beginning at its first appearance when the temperature of the hydrosphere was too high for it to have contained dissolved oxygen; only at a later period, when the temperature became low enough for the water to contain oxygen in solution, did some of the forms begin

[17] Mereschowsky, C., "Theorie der zwei Plasmaarten als Grundlage der Symbiogenesis, einer neuen Lehre von der Entstehung der Organismen," *Biol. Centralblatt*, XXX, 1910, pp. 278–303, 321–347, 353–367.

[18] The term mycoplasm used by Mereschowsky must not be confounded with the similar word used by Eriksson and other botanists in reference to the manner in which Rust-Fungi permeate their hosts.

[19] In the first epoch the earth was an incandescent mass of vapor; in the second it had a firm crust, but the temperature was far too high to permit of the condensation of water-vapor upon its surface.

to adapt themselves to these conditions, and became secondarily facultative or obligate aerobes. Amœboplasm, on the other hand, can not exist without a supply of oxygen. (2) Mycoplasm can support temperatures of 90° C. or even higher; amœboplasm can not support a temperature higher than 45° C. or 50° C. (3) Mycoplasm is capable of building up albumins and complex organic substances from inorganic materials; amœboplasm is incapable of doing so, but requires organic food. (4) Mycoplasm has restricted powers of locomotion and is incapable of amœboid movement, or of forming the contractile vacuoles seen commonly in amœboplasm. (5) Mycoplasm, in contrast to amœboplasm, is rich in phosphorus and nuclein. (6) Mycoplasm is extraordinarily resistant to poisons and utilizes as food many substances that are extremely deadly to amœboplasm, such as prussic acid, strychnine and morphia. (7) Amongst minor differences, mycoplasm is characterized by the presence of iron in the combined state and possesses a far more complicated structure than amœboplasm, a peculiarity which enables mycoplasmic cell-elements (chromosomes) to function as the bearers of hereditary qualities.

The course of the evolution of living beings, according to Mereschkowsky, was as follows. The earliest forms of life were "Biococci," minute ultra-microscopic particles of mycoplasm, without organization, capable of existing at temperatures near boiling-point and in the absence of oxygen, possessing the power of building up proteins and carbohydrates from inorganic materials, and very resistant to strong mineral salts and acids and to various poisons. From the Biococci arose in the first place the Bacteria, which for a time were the only living inhabitants of the earth. Later, when the temperature of the terrestrial waters had been lowered below 50° C., and contained abundant organic food in the shape of Bacteria, amœboplasm made its appearance in small masses as non-nucleated Monera which crept in an amœboid manner on the floor of the ocean and devoured Bacteria.

The next step in evolution is supposed to have been that, in some cases, micrococci ingested by the Monera resisted digestion by them and were enabled to maintain a symbiotic existence in the amœboplasm. At first the symbiotic micrococci were scattered in the Moneran body, but later they became concentrated at one spot, surrounded by a membrane, and gave rise to the cell-nucleus. In this way, by a "symbiogenesis" or process of symbiosis between two distinct types of organisms, Mereschkowsky believes the nucleated cell to have arisen, an immense step forward in evolution, since the locomotor powers of the simple and delicate Monera were now supplemented by the great capability possessed by the Bacteria of producing ferments of the most varied kinds.

Meanwhile it is supposed that the free Bacteria continued their natural evolution and gave rise to the Cyanophyceæ, and to the whole group of Fungi. The plant-cell came into existence by a further process of symbiogenesis, in that some of the Cyanophyceæ, red, brown or green in color, became symbiotic in nucleated cells, for the most part flagellates, in which they established themselves as the chromatophores or chlorophyll-corpuscles. In this way Mereschkowsky believes the vegetable cell to have come into existence, and the evolution of the Vegetable Kingdom to have been started, as a double process of symbiosis. Those amœboid or flagellated organisms, on the other hand, which formed no symbiosis with Cyanophyceæ, continued to live as animals and started the evolution of the Animal Kingdom.

As a logical deduction from this theory of the evolution of living beings, Mereschkowsky classifies organisms generally into three groups or Kingdoms: first the Mycoidea, comprising Bacteria, Cyanophyceæ, and Fungi, and in which no symbiosis has taken place; secondly, the Animal Kingdom, in which true cells have arisen by a simple symbiosis of mycoplasm (chromatin) and amœboplasm (cytoplasm); thirdly, the Vegetable Kingdom, in which true

cells have entered upon an additional symbiosis with Cyanophyceæ, chromatophores or chlorophyll-corpuscles.

Interesting and suggestive as are the speculations of Mereschkowsky, they are nevertheless open to criticism from many points of view. I will not enter here into criticisms which I regard as beyond my competence. It is for botanists to pronounce upon the notion that Bacteria, Cyanophyceæ, and Fungi can be classified together as a group distinct from all other living beings; to decide whether the protoplasm of the Cyanophyceæ and Fungi can be regarded as consisting of mycoplasm alone, and not of a combination of nuclei and cytoplasm, such as is found in true cells and represents, according to Mereschkowsky, a symbiosis of mycoplasm and amœboplasm. I think I am right in saying that botanists are agreed in regarding Fungi as derived from green algæ, and as possessing nuclei similar to those of the higher plants. As a zoologist the point that strikes me most is the absence of any evidence that true Monera, organisms consisting of cytoplasm alone, exist or could ever have existed. Mereschkowsky supposes that when the Monera came into being they maintained their existence by feeding upon Bacteria. In order to digest Bacteria, however, the Monera must have been capable of producing ferments, and therefore did not acquire this power only as the result of symbiosis with Bacteria, unless it be assumed that the symbiosis came about at the instant that amœboplasm came into existence. There is, however, no evidence that cytoplasm by itself can generate ferments. All physiological experiments upon the digestion of Protozoa indicate that the cytoplasmic body, deprived of the nucleus, can not initiate the digestive process. Consequently the existence of purely cytoplasmic organisms would seem to be an impossibility.

For my part, I am unable to accept any theory of the evolution of the earliest forms of living beings which assumes the existence of forms of life composed entirely of cytoplasm without chromatin. All the results of modern

investigations into the structure, physiology and behavior of cells on the one hand, and of the various types of organisms grouped under the Protista, on the other hand—the combined results, that is to say, of cytology and protistology—appear to me to indicate that the chromatin-elements represent the primary and original living units or individuals, and that the cytoplasm represents a secondary product. I will summarize briefly the grounds that have led me to this conviction, and will attempt to justify the faith that I hold; but first I wish to discuss briefly certain preliminary considerations which seem to me of great importance in this connection.

It is common amongst biologists to speak of "living substance," this phrase being preceded by either the definite or the indefinite article—by either "the" or "a." If we pause to consider the meaning of the phrase, it is to be presumed that those who make use of it employ the term "substance" in the usual sense to denote a form of matter to which some specific chemical significance can be attached, which could conceivably be defined more or less strictly by a chemist, perhaps even reduced to a chemical formula of some type. But the addition of the adjective "living" negatives any such interpretation of the term "substance," since it is the fundamental and essential property of any living being that the material of which it is composed is in a state of continual molecular change and that its component substance or substances are inconstant in molecular constitution from moment to moment. When the body of a living organism has passed into a state of fixity of substance, it has ceased, temporarily or permanently, to behave as a living body; its fires are banked or extinguished. The phrase "living substance" savors, therefore, of a *contradictio in adjecto;* if it is "living" it can not be a "substance," and if it is a "substance" it can not be "living."

As a matter of fact, the biologist, when dealing with purely biological problems, knows nothing of a living substance or substances; he is confronted solely by living in-

dividuals, which constitute his primary conceptions, and the terms "life" and "living substance" are pure abstractions. Every living being presents itself to us as a sharply-limited individual, distinct from other individuals and constituting what may be termed briefly a microcosmic unit, inasmuch as it is a unity which is far from being uniform in substance or homogeneous in composition, but which, on the contrary, is characterized by being made up of an almost infinite multiplicity of heterogeneous and mutually interacting parts. We recognize further that these living individuals possess invariably specific characteristics; two given living individuals may be so much alike that we regard them as of the same kind or "species," or they may differ so sharply that we are forced to distinguish between them specifically. Living beings are as much characterized by this peculiarity of specific individuality as by any other property or faculty which can be stated to be an attribute of life in general, and this is true equally of the simplest or the most complex organisms; at least we know of no form of life, however simple or minute, in which the combined features of individuality and specificity are not exhibited to the fullest extent. A living organism may be so minute as to elude direct detection entirely by our senses, even when aided by all the resources furnished by modern science; such an organism will, nevertheless, exhibit specific properties or activities of an unmistakable kind, betraying its presence thereby with the utmost certainty. The organisms causing certain diseases, for example, are ultra-microscopic, that is to say, they have not been made visible as yet, and an exact description or definition can not be given of them at the present time; yet how strongly marked and easily distinguishable are the specific effects produced by the organisms causing, respectively, measles and small-pox, for instance, each, moreover, remaining strictly true and constant to its specific type of activity; the organism, whatever its nature may be, which causes measles can not

give rise to small-pox, nor *vice versa*, but each breeds as true to type as do lions and leopards.

The essential and distinctive characteristic of a living body of any kind whatsoever is that it exhibits while it lives permanence and continuity of individuality or personality, as manifested in specific behavior, combined with incessant change and lability of substance; and further, that in reproducing its kind, it transmits its specific characteristics, with, however, that tendency to variability which permits of progressive adaptation and gradual evolutionary change. It is the distinctively vital property of specific individuality combined with "stuff-change" (if I may be allowed to paraphrase a Teutonic idiom) which marks the dividing line between biochemistry and biology. The former science deals with substances which can be separated from living bodies, and for the chemist specific properties are associated with fixity of substance; but the material with which the biologist is occupied consists of innumerable living unit-individuals exhibiting specific characteristics without fixity of substance. There is no reason to suppose that the properties of a given chemical substance vary in the slightest degree in space or time; but variability and adaptability are characteristic features of all living beings. The biochemist renders inestimable services in elucidating the physico-chemical mechanisms of living organisms; but the problem of individuality and specific behavior, as manifested by living things, is beyond the scope of his science, at least at present. Such problems are essentially of distinctively vital nature and their treatment can not be brought satisfactorily into relation at the present time with the physico-chemical interactions of the substances composing the living body. It may be that this is but a temporary limitation of human knowledge prevailing in a certain historical epoch, and that in the future the chemist will be able to correlate the individuality of living beings with their chemico-physical properties, and so explain to us how living beings first came into existence; how, that is to say, a combination of chemical

substances, each owing its characteristic properties to a definite molecular composition, can produce a living individual in which specific peculiarities are associated with matter in a state of flux. But it is altogether outside the scope and aim of this address to discuss whether the boundary between biochemistry and biology can be bridged over, and if so, in what way. I merely wish to emphasize strongly that if a biologist wishes to deal with a purely biological problem, such as evolution or heredity, for example, in a concrete and objective manner, he must do so in terms of living specific individual units. It is for that reason that I shall speak, not of the chromatin-substance, but of chromatinic elements, particles or units, and I hope that I shall make clear the importance of this distinction.

To return now to our chromatin; I regard the chromatinic elements as being those constituents which are of primary importance in the life and evolution of living organisms mainly for the following reasons: the experimental evidence of the preponderating physiological *rôle* played by the nucleus in the life of the cell; the extraordinary individualization of the chromatin particles seen universally in living organisms, and manifested to a degree which raises the chromatinic units to the rank of living individuals exhibiting specific behavior, rather than that of mere substances responsible for certain chemico-physical reactions in the life of the organism; and last, but by no means least, the permanence and, if I may use the term, the immortality of the chromatinic particles in the life-cycle of organisms generally. I will now deal with these points in order; my arguments relate, in the first instance, to those organisms in which the presence of true cell-nuclei renders the identification of the chromatin-elements certain, as pointed out above, but if the arguments are valid in such cases they are almost certainly applicable also to those simpler types of organisms in which the identification of chromatin rests on a less secure foundation.

The results obtained by physiological experiments with

regard to the functions of the nuclear and cytoplasmic constituents of the cell are now well known and are cited in all the text books. It is not necessary, therefore, that I should discuss them in detail. I content myself with quoting a competent and impartial summary of the results obtained:

> A fragment of a cell deprived of its nucleus may live for a considerable time and manifest the power of coordinated movements without perceptible impairment. Such a mass of protoplasm is, however, devoid of the powers of assimilation, growth, and repair, and sooner or later dies. In other words, those functions that involve destructive metabolism may continue for a time in the absence of the nucleus; those that involve constructive metabolism cease with its removal. There is, therefore, strong reason to believe that the nucleus plays an essential part in the constructive metabolism of the cell, and through this is especially concerned with the formative processes involved in growth and development. For these and many other reasons . . . the nucleus is generally regarded as a controlling centre of cell-activity, and hence a primary factor in growth, development, and the transmission of specific qualities from cell to cell, and so from one generation to another.[20]

I may add here that the results of the study of life-cycles of Protozoa are entirely in harmony with this conception of the relative importance of nuclear—that is chromatinic—and cytoplasmic cell-constituents, since it is not infrequent that in certain phases of the life-cycle, especially in the microgamete-stages, the cytoplasm is reduced, apparently, to the vanishing point, and the body consists solely of chromatin, so far as can be made out. In not one single instance, however, has it been found as yet that any normal stage in the developmental cycle of organisms consists solely of cytoplasm without any particles of chromatin.

While on the subject of physiological experiment, there is one point to which I may refer. Experiments so far have been carried on with Protozoa possessing definite nuclei. It is very desirable that similar experiments should be conducted with forms possessing chromidia in addition to nuclei, in order to test the physiological capa-

[20] E. B. Wilson, "The Cell," second edition, 1911, pp. 30 and 31.

bilities of chromatin-particles not concentrated or organized. *Arcella* would appear to be a very suitable form for such investigations. This is a point to which my attention was drawn by my late friend Mr. C. H. Martin, who has lost his life in his country's service.

I have mentioned already in my introductory remarks that the only reliable test of chromatin is its behavior, and the whole of modern cytological investigation bears witness to the fact that the chromatinic particles exhibit the characteristic property of living things generally, namely, individualization combined with specific behavior. In every cell-generation in the bodies of ordinary animals and plants the chromatin-elements make their appearance in the form of a group of chromosomes, not only constant in number for each species, but often exhibiting such definite characteristics of size and form, that particular, individual chromosomes can be recognized and identified in each group throughout the whole life-cycle. Each chromosome is to be regarded as an aggregate composed of a series of minute chromatinic granules or chromioles, a point which I shall discuss further presently. Most striking examples of the individualization of chromosomes have been made known recently by Dobell and Jameson[21] in Protozoa. Thus in the Coccidian genus *Aggregata* six chromosomes appear at every cell-generation, each differing constantly in length if in the extended form, or in bulk if in the contracted form, so that each of the six chromosomes can be recognized and denoted by one of the letters *a* to *f* at each appearance, *a* being the longest and *f* the shortest.

(*To be continued.*)

[21] *Proc. Roy. Soc.* (B), Vol. 89. (In the press.)

THE EUGSTER GYNANDROMORPH BEES

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About fifty years ago von Siebold wrote his classic paper on "Zwitterbienen" in which he gave an account of anomalous bees that appeared in considerable numbers in a hive of a bee breeder, named Eugster, in Constance.[1] The particular interest that attached to the case was not only that a bee might be partly male and partly female, mixed in all manner of proportions, but that they were hybrid bees as well, the mother belonging to the race of Italian bees, while the father or fathers were German bees. Von Siebold did not state in his paper whether the male parts of the gynandromorph were like the father, or were hybrid, or were like the mother. In fact it was not until 1888 that the importance of such information was realized. In that year Boveri described a result that he had obtained with the eggs of the sea urchin, in which as a result of delayed fertilization (or of some irregularity in the penetration of the sperm into the egg) the sperm nucleus fused with one of the two nuclei resulting from the division of the egg nucleus. In consequence half of the nuclei were derived from the egg alone, while the other half of the nuclei arose from the union of the paternal and a maternal nucleus. If now, as other evidence seemed to show, one nucleus in the bee produces a male and two nuclei a female, such a partially fertilized egg should be male on one side and female on the other side of the body of the resulting individual. In this way, Boveri pointed out, the Eugster gynandromorphs might have arisen.

In 1905 I pointed out that the Eugster gynandromorphs might also be accounted for by means of another hypothesis. If two (or more) spermatozoa should enter the egg, one of them might unite with the egg nucleus while the

[1] Several earlier accounts of gynandromorph bees are extant (See "literature" list).

other might give rise to the nuclei of the rest of the embryo. On this hypothesis the combined nuclei would give rise to the female parts, while the single nucleus, here derived from the sperm, would give rise to the male parts. In support of such a view I pointed out that more than a single nucleus was known to enter the egg of the bee, and this condition has more recently been amply confirmed by Nachtsheim. I also pointed out, for the first time I believe, that a decision in favor of one or the other of these two hypotheses could be obtained if in these gynandromorph hybrids the nature of the male and of the female parts of the adult were known; for on Boveri's interpretation the male parts (derived from the single egg nucleus) should be maternal while on my view the male parts (derived from the single sperm nucleus) should be paternal. In both views the female parts of the gynandromorphs should be hybrid and therefore either intermediate in character or like the dominant strain.

Four years ago Professor Doflein looked through the collection at Munich, at the request of Boveri, to find out whether any of the Eugster bees were still preserved there, and luckily found a jar labelled "Apis Mellifica, Zwitterbienen" which turned out to be the bees that von Siebold had obtained. Owing to their long sojourn in alcohol the color was almost entirely gone and on the color depended the decision as to the difference between the two races that combined to produce the gynandromorphs. At first Boveri despaired of finding out from these alcoholic specimens whether the male parts were like the father or like the mother; but on cleaning the parts he found that he could still determine whether a part was more like the same part in one or in the other domesticated strain.

Briefly Boveri finds that the male parts of the gynandromorphs are maternal, while the female parts are paternal, which is the dominant character. This conclusion gives a decisive answer in favor of his hypothesis and sets my own aside for this case at least.

Boveri's evidence leaves no reasonable doubt as to the possibility of determining the nature of the character of

the gynandromorphs, yet the desirability of having it confirmed on living material may be still worth while, since, as Boveri points out in a postscript, von Engelhardt has recently (1914) described some hybrid gynandromorphs from fresh material which lead to the opposite conclusion from that to which Boveri has arrived. Von Engelhardt's bees arose from an Italian queen by a "domestic" drone. Until it is ascertained what variety was used as the domestic drone the value of the evidence is not entirely certain.

A student of Boveri's, Fr. Elsa Mehling, has made a very careful study of the Eugster gynandromorphs, paying attention to a number of characters. Her work adds many details of interest concerning the admixture of male and female parts, but does not, however, furnish much additional evidence concerning the origin of these parts. She arrives at the same conclusion as that reached by Boveri, viz., that the male parts are maternal.

In this connection it should be recalled that the long sought for evidence demonstrating that drones inherit the characters of their mother has at last been found by Newell. Working at an isolated station forty miles from Houston, Texas, he mated Italian and Carniolan races of bees. The Italians are distinctly yellow, while the Carniolans are more or less gray. The stocks used had been under observation for several generations and were known to be pure. When virgin Italian queens were mated to Carniolan drones the workers and queens (both of which come from fertilized eggs) are like the Italian yellow stock, which is, therefore, dominant as to color. The drones from this mating are also yellow, which is expected if they inherit from their mother, but the cross made this way is not decisive in regard to the inheritance of the drones, because the maternal color is here dominant. In the reciprocal cross the result is decisive. Thus when a Carniolan queen is mated to an Italian drone the workers and queens are yellow due to the dominant color of the father, but the drones are gray like the pure Carniolan drones. This result proves that the characters of the

drones come from the mother, which is in accord with Dzierzon's theory that the drones arise from unfertilized eggs. This is further established by the following evidence. The daughters (queens) that come from Italian queens by Carniolan drones give rise to two kinds of drones in equal numbers, viz., Italian and Carniolan, which is the expected result, since such daughters are hybrid and are expected to produce two kinds of eggs. Reciprocally also the daughters from Carniolan queens by Italian drones produce two kinds and only two kinds of drones in equal numbers. The result also shows that Mendel's law applies to the queen bee. Cuénot has recently recorded the appearance of some drones in hybrid hives that are intermediate or even like the father, but since the possible production of drones by hybrid workers was not excluded, at least so far as the published evidence goes, these sporadic cases can not be used to disprove the maternal inheritance of the drones.

Boveri has discussed certain cytological possibilities in relation to the gynandromorph bees that are of interest. His work, and that of Herbst on sea-urchin embryos, had shown that haploid nuclei have only half the volume of diploid nuclei. It might have been anticipated therefore that the nuclei (and cells) of the drone bee would be half the size of those of the queen or of the worker bee, but a study of the cells of drones by Oeninger had already shown that their nuclei are as large as are those of the workers which have the diploid number of chromosomes. It is not possible therefore to determine by microscopic study of nuclear size whether or not the male parts of gynandromorphs come from a single nucleus.

Boveri points out that, since the nucleus of the egg of the bee, if not fertilized, proceeds to divide, it is improbable that the division center is brought in by the sperm, as appears to be the case in so many other eggs. Nachtsheim's observations confirm, he believes, this interpretation in the bee; for, according to Nachtsheim, three to seven or more nuclei enter but only one of these fuses with the egg nucleus. The others move out into the egg,

their chromosomes are resolved, and a spindle develops. But these spindles lack centrioles at their poles. The mitotic figure that has reached this stage then proceeds to degenerate. The absence of the centrioles indicates, Boveri thinks, that the spermatozoa of the bee does not bring in a division center, hence this cell organ must be contributed by the egg, and in consequence we can now easily understand how facultative parthenogenesis is, so to speak, a normal phenomenon in this egg. Boveri does not point out however that Nachtsheim's figures show that the polar spindles of the bee's egg also lack centrioles, and yet mitotic division is accomplished. It seems highly questionable therefore whether much weight is to be attached to the absence of centrioles in the supernumerary sperm figures. The chief interest that attaches to Boveri's argument is his disclaimer that he intended his striking statement in regard to fertilization, namely, that the sperm furnishes the dynamic division center for development, to be taken as a universal dictum. The incitement of artificial division centers in such eggs as those of the sea urchin in which the sperm brings in the centriole (or causes its development in the immediate vicinity of the sperm nucleus) shows how little importance can be attached to the hypothesis of the genetic continuity of the centrosome. If in the case of the bee three or more sperm enter each egg all bees would be gynandromorphs should all the sperm develop. Obviously, some special condition must be assumed to be present if these sperms are to go forward and complete their development which they begin even under ordinary circumstances. Boveri himself must also invoke some special condition, such as retarded fertilization, in order that one of the entering sperm fuses with one of the products of the first division of the egg nucleus. It might equally well be postulated that delay in the fertilization and the consequent impetus to parthenogenesis might be favorable for the completion of the division of the supernumerary asters. In a word it is doubtful if Boveri's interpretation gains much from his cytological argument. If his observations on the dis-

tribution of color are well established this further argument is superfluous.

In 1906 Toyama described a gynandromorph that arose when two races of silkworm moths were crossed. From an analysis of the genetic evidence I pointed out that in this case the male parts of the gynandromorph must have been paternal and the hybrid parts maternal (dominant). If the same conditions prevail here as in the bee, viz., one nucleus producing a male and two producing a female,[2] the case is in harmony with my hypothesis and not with that of Boveri. But the evidence for my view is not as strong as that Boveri's is now for the bee; yet it may be true, nevertheless, that in both of these ways gynandromorphs may arise. A third mode of origin has been shown, from the genetic evidence, to apply to *Drosophila*, viz., dislocation during ontogeny of the two sex chromosomes. In fact we should expect that gynandromorphs would arise in insects whenever certain nuclei come to contain two sex chromosomes and others only one. The means by which this segregation takes place may differ under different conditions.

Goldschmidt has recently explained the remarkable gynandromorphs that he obtains in crosses between *Lymantria dispar* and *L. japonica* in still a different way, one that involves the relative potencies of the sex factors in the different races.

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[2] Whether one is justified in applying to the case of the moth the hypothesis for the bee may be seriously questioned because in the case of the moth the male is assumed to be the result of one sex chromosome (z) in conjunction with the *haploid* number of autosomes, while in the female moth one sex chromosome (z) and its mate (w) (which from Doncaster's evidence has no sex-determining influence) in conjunction with the *diploid* number of autosomes is assumed to stand for the female soma.

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SHORTER ARTICLES AND DISCUSSION

PINK-EYED WHITE MICE, CARRYING THE COLOR FACTOR

AMONG the many domesticated varieties of the house mouse (*Mus musculus*), two sorts with entirely white pelage are known, —the albino, and the black-eyed white. Numerous experiments have shown that the albino differs from colored varieties by the loss of a single factor, the color factor; for, in crosses with colored varieties, albinism acts as a recessive allelomorph. The genetic composition of the black-eyed white is less well known although several hypotheses have been suggested. Black-eyed whites possess the color factor as crosses with albinos have shown. They may be homozygous in the factor for dark eyes. A black-eyed white male produced 189 dark-eyed offspring in my experiments when mated to pink-eyed intense brown females. The offspring of this cross were heterozygous in dark eye (Dd). By mating them inter se, pink-eyed forms were obtained in the F_2 generation, some of which had a pure white coat. In other words, it is possible to recombine the factors producing the pure white pelage of the black-eyed whites with the pink-eyed condition. Such pink-eyed whites resemble true albinos in appearance, but not in zygotic constitution, for they still retain the color factor although they show no color. To avoid confusion in discussion, I shall refer to this synthesized form of albino as a pink-eyed white to distinguish it from the albino lacking the color factor. Predictions often compel subsequent retractions; however, I feel safe in predicting colored offspring from a cross between the pink-eyed white and the albino, although externally the mating resembles a cross between albinos which always breed true. Black-eyed white strains sometimes show a few colored hairs around the ears, between the eyes, and in front of the tail. The corresponding pink-eyed white forms may also show the same characteristic.

The white coat and pink eyes of the albino mouse are due to the loss of a single factor; but the white coat of the black-eyed white strains cannot be accounted for in such a simple manner. Little ('13) seemed inclined to the view that the black-eyed white mouse was a spotted individual in which the spotting was of the

recessive type, in contradistinction to spotting of the dominant type described by Miss Durham ('08). Through the kindness of Professor W. E. Castle, a black-eyed white male was received in the fall of 1914. With this male it was possible to produce other black-eyed whites. In such black-eyed whites as I have been able to test, both dominant and recessive spotting were present. Furthermore, the recessive spotting always occurred in double dose. Hence, black-eyed whites were supposed to have the zygotic formula PPss or Ppss, in which P stands for dominant spotting and p for its absence; and s represents the factor for recessive spotting which is allelomorphic to self (S). So far, I have been able to test sufficiently only two black-eyed white males, both of which were clearly of the formula Ppss. When mated to self-colored females they gave 231 offspring. Since these offspring showed much variability, they were graded in classes ranging from self (—9) to black-eyed white (+9) according to the amount of pigmentation which they showed. A distinct grouping around two modes was found as follows:

Class modes..	−9	−8	−7	−6	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9
Frequencies..	98	24	8	16	33	26	8	4	6	2	3	1	1	1	0	0	0	0	0

About one-half of the F_1 offspring was grouped around the lower mode (126), and the other half (105) grouped around the upper mode, if we assume the class —7, as the dividing class. Very few individuals were found in the "doubtful class." Expressing the cross of black-eyed white with self-colored in Mendelian terms, it would be:

$$\begin{array}{ll} \text{Ppss} \times \text{ppSS} = P_1 \text{ zygotes} \\ \text{Ps} + \quad \text{ps} = \text{gametes of black-eyed white } P_1 \\ \text{pS} + \quad \text{pS} = \text{gametes of self-colored } P_1 \\ \hline \text{PpSs} + \text{ppSs} = F_1 \text{ zygotes} \\ \text{Spotted} + \text{Self} \end{array}$$

The results conformed to this expectation. The individuals grouped around the lower mode were self-colored or very nearly so, as one would expect of individuals heterozygous in self and recessive spotting, for self is dominant or very nearly dominant to recessive spotting. Their formula was ppSs. Subsequent experiments corroborated this, for they produced self and recessive spotted in Mendelian ratios, when mated inter se or to recessive spotted individuals. They never gave black-eyed whites in

such matings. Those offspring grouped around the upper mode were spotted, and had a formula PpSs. When mated inter se or back to recessive spotted, they gave, besides spotted and selfs, black-eyed whites; apparently because the combination Ppss could again be formed. The dominant spotting factor, P, evidently acts more vigorously upon recessive spotting than upon self. It can not restrict the more extended pigmentation of a self coat completely. Hence, half of the F_1 individuals (those with the formula PpSs) were spotted, or, to describe them more accurately, spotted with frequent and varying amounts of silvering. The dominant spotting factor, P, can, however, restrict the limited pigmentation of a recessive spotted coat completely or almost completely. Hence animals with the formula Ppss were black-eyed whites.

The origin of our new pink-eyed white forms, which resemble albinos so closely as to be indistinguishable from them, is evidently due to the substitution of the pink-eye factor for dark-eye in black-eyed whites, and not due to the loss of the color factor C. In our cultures, the black-eyed whites have the formula PpssDDCC and the corresponding pink-eyed whites had the formula PpssddCC where D and d represent dark eye and pink eye respectively, and C represents the color factor. We have also produced black-eyed white forms heterozygous in dark eye, PpssDd. Black and brown are likewise interchangeable in the dark-eyed whites, for black-eyed whites, heterozygous in black, have been produced. I see no reason why brown-eyed whites can not be produced in the usual Mendelian fashion by mating black-eyed whites to browns, and recovering the white pelage with brown eyes in the F_2 generation. Mating the spotted F_1 offspring inter se should give, among others, individuals with the formula PpssbbCC. These would be brown-eyed whites,—white because of the combined action of P and s, and brown simply because they lack the differential factor B which changes brown into black.

The occurrence of pink-eyed whites which resemble albinos may have some bearing on an anomalous case cited by Bateson ('04) as follows: "the production of colored animals by albinos, is not, so far as I know, illustrated by a single case, with the following exception. In the later editions of "Fancy Mice" (Upcott Gill), Dr. Carter Blake, formerly secretary of the Anthropological Institute commenting on the statement that albino mice of whatever

parentage produce nothing but albinos, writes that a pair of albinos produced some brown-and-white, some plum, some grey, and some albinos. If this result occurred under all precautions, it stands alone.'' Allen ('04) attempted to account for this case by postulating an error in recording the true sire, or that the animals used were not true albinos but black-eyed whites. That two individuals having white coats and pink eyes can give colored young is perfectly possible. The pink-eyed whites in my cultures have a white pelage because of the combined effect of the dominant and recessive spotting, while their pink eye is due to the loss of the dark-eye factor. They still retain the color factor, although they show no color. They may be called albinos, if we define an albino as any pink-eyed white individual; but they should be carefully distinguished from that type of albinism which is due to the loss of the color factor. If we mate these two different types of albinos together, we should obtain colored young. The cross may be expressed in symbols:

PpssddCC × ppSSDDcc	.... P. zygotes
PsdC + psdC	.... gametes of pink-eyed white
pSDc + pSDc	.... gametes of albino
PpSsDdCc + ppSsDdCc	.... F_1 zygotes
Spotted + Selfs	

It is interesting to note that the exceptional case, quoted by Bateson, mentions the occurrence of spotted and selfs in the cross of two albinos. In plants, as in animals, similar somatic characters do not necessarily indicate similar germinal constitution.

Our assumption of the interaction of a dominant and recessive spotting factor to account for the white pelage of pink-eyed and black-eyed whites is strengthened by the valuable paper of Little ('15). Little has adopted a similar hypothesis for black-eyed whites in his paper just published, and quite different from the hypothesis of his earlier paper ('13). It should be stated that Little's experiments furnish even a larger amount of data from the more convincing type of matings than has been possible in our own cultures as yet.

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PARTHENOGENESIS AND SEXUAL REPRODUCTION IN ROTIFERS. EXPERIMENTAL RESEARCH UPON BRACHIONUS PALA[1]

In a recent number of *Bios* Miss Lina Moro has presented some interesting and suggestive results from experiments upon the rotifer, *Brachionus pala*. She has subjected the parthenogenetic females to various chemicals, to changes in nutrition, and to changes in temperature.

In using $FeCl_3$ solutions she has been able to produce male-producing females in small numbers while in control experiments in which no $FeCl_3$ was used no male-producing females were produced. Many dilutions of $FeCl_3$ were used but *M*/12,000 seemed to be the optimum dilution. This was added to the culture water of hay infusion in which the rotifers were living. Although the number of the experiments were rather small and the percentage of male-producing females obtained was not higher than 12 per cent., nevertheless they indicate the possibility of a specific chemical being able to induce the production of male-producing females.

Not only did $FeCl_3$ cause male-producing females to appear but it also caused the mothers to form the eggs much faster in their bodies and to extrude them to the outside much faster than those in the controls. Usually while a female in the control was producing one egg a female in the $FeCl_3$ would produce four eggs. This rapid formation and production of eggs after it was once started continued through many subsequent generations during the three months in which the experiments were carried on. It might be considered that this new characteristic induced by a chemical was a case of the formation of a new character which, after it was once formed, was inherited by the descendants.

It was also determined that the influence of the $FeCl_3$ acted upon the egg while it was yet inside the mother and caused it to develop into a male-producing female. After the egg was laid its development could not be altered from a female-producing female to a male-producing female by the use of $FeCl_3$.

A dilution of $HgCl_2$ (*M*/1,200,000,000) was also effective in causing male-producing females to appear but a smaller number of offspring were produced than in the $FeCl_3$. The percentage

[1] "Partenogenesi e Anfigonia nei Rotiferi. Recerche sperimentali sul *Brachionus pala*," by Lina Moro, *Bios*, Vol. 2, Fasc. 3, pp. 219–264, 1915.

of male-producing females produced was about 18 per cent. It also caused an increase in the number and the rate of production of the eggs by each female as was the case in the $FeCl_3$ experiments. KCl (M/12,000) in the very few experiments recorded caused about 16 per cent. of male-producing females to appear and $CaCl_2$ (M/12,000) caused about 33 per cent. of male-producing females to appear. In the controls for these experiments no male-producing females appeared. In all of these chemical experiments each mother after being transferred from the control to the culture media containing the various chemicals produced a family of several daughters but in each family there was never more than *one* male-producing daughter. In the $FeCl_3$ solutions each mother produced many daughters and as only one of them in each family was a male-producer the percentage of male-producing females was necessarily lower whereas in the $HgCl_2$, KCl, $CaCl_2$, solutions each mother produced fewer daughters than in the $FeCl_3$ solutions, and as only one of these in each family was a male-producer, the percentage of male-producing females was consequently higher. Various dilutions were used of $AlCl_3$, KCN, NaCl, Na_2HAsO_4, HCl, and NaOH but none of them caused male-producing females to appear.

In the nutrition experiments it was found that a constant diet at a uniform temperature of 15° C.–17° C. or 25° C.–27° C. produced only female-producing females but in some experiments in which an abundance of food was used for a time and then was followed by a period of scanty food or semi-starvation many male-producing females appeared, especially at the lower temperature.

In some of the experiments a temperature of 15° C.–17° C. produced all female-producing females but when the mothers were put at a temperature of 25° C.–27° C. or at 31° C. as high as 50 per cent. of the daughters were male-producers. When these same mothers were transferred back to 15° C.–17° C. they again produced only female-producing daughters. In a few experiments at a constant temperature of 25° C.–27° C. only female-producing females were produced but when the mothers were put at a lower temperature they produced many male-producing daughters. The general conclusion drawn is that whenever the general cultural conditions are constant and uniform, whether they refer to nutrition or to temperature, only female-producing females are produced but when the cultural conditions are sud-

denly changed by the disappearance of an abundant diet or by the rise or fall in the temperature male-producing females are produced at once. In a few experiments very young females (1–7 hours after hatching) were put from a high temperature to a temperature of 9° C.–11° C. and many of them developed into male-producers but whether this was due to the temperature or to some other factor was not known.

Another fact of considerable interest was verified. It concerned the nature of the male-producing females and the sexual females (the females which produce fertilized eggs). It has been observed by several investigators that if the small male eggs of a male-producing female are fertilized, in a species of *Asplancha* and *Hydatina senta,* they develop into the winter or resting eggs. This was found to be true also in *Brachionus pala.*

In all the families of daughters from the various mothers it was found that the male-producing daughters were among the earliest ones produced of each family. This was observed in the families of *Hydatina senta* by an earlier worker but later it was found to be due entirely to the method of feeding.

Although, as stated previously, the observations recorded in this paper are from a rather small number of individuals and ought to be expanded and verified, nevertheless, they show that in this rotifer the production of female-producing or male-producing females can be regulated by the environment and thus the results are in a general accord with the observations obtained by several workers with the rotifers, *Asplancha,* and *Hydatina senta.*

D. D. Whitney

NOTES AND LITERATURE

AN OUTLINE OF CURRENT PROGRESS IN THE THEORY OF CORRELATION AND CONTINGENCY

WORKERS in the physical sciences realized long ago that certain progress depended upon the precision of their instruments of measurement and the adequacy of their methods of mathematical description and analysis. Biologists, here and there, are beginning to see the importance of the analytical as well as of the observational tools. Among the analytical formulæ none are of greater usefulness than those for measuring interdependence. It may not be out of place, therefore, to sketch in simple terms for the benefit of those who are interested in the methods only as a means to an end, the progress which is being made in the perfection of these instruments of research.

The term *current* as used in these paragraphs is made more comprehensive than is conventional; some of the citations are four or even more years old. The elasticity of the term is justified in dealing with the literature of a field in which progress is particularly difficult and in which actual contributions are incorporated but slowly into the working technique of the biologist. Indeed, biologists as a class still think of correlation as synonymous with the classical product-moment method. How erroneous this impression is will appear in the following pages.

The purpose of this review is therefore to indicate in non-mathematical terms easily comprehensible to biological readers the lines of advances in the theory of the measurement of interdependence in order that they may the more easily select for dealing with their actual data, formulæ of the existence of which they might otherwise be unaware.

The progress which we have to consider has been along four different lines:

(*a*) In the simplification of methods of computation in the case of familiar formulæ. (*b*) In the development of entirely new formulæ applicable to data of particular sorts. (*c*) In the determination of the corrections to be applied for grouping into "broad categories." (*d*) In partial correlation, multiple correlation, and the correlation of indices and increments.

In this review we shall limit ourselves strictly to an outline of progress which has been made in the theory of the measurement of the interrelationship of two variates, leaving for consideration at a later time the far more complex subjects of correction for grouping, partial and multiple correlation, variate difference correlation and some other topics.

The detailed advances may be most easily understood by considering the kinds of data with which one has to deal in determining the degree of interdependence, association or correlation (to use these terms in a broad sense) between two variates.

An arrangement of the literature according to a key similar to that familiar in taxonomic works will perhaps be of service to the biologist who desires to locate at once the literature pertinent to the particular kind of data with which he has to deal.

Suppose first of all that the two characters are both suitable for measurement (or counting) on a quantitative scale and that for both the measurements form several classes. The choice of methods for measuring the correlation between them will then depend upon whether the average values of the y character associated with serially arranged values of the x character lie in sensibly a straight line or whether they can best be represented by some more complex curve. Linearity of regression, as it is technically called, has therefore a two-fold significance. (*a*) Biologically, it shows that an associated character changes at a *uniform* rate (however slight this rate may be) with the variation of a selected character. (*b*) Statistically, it justifies the application of the familiar product-moment method of determining the correlation coefficient.

Both Characters Measurable on a Quantitative Scale, Regression Linear.—So satisfactory has the product-moment method proved for data in which both characters are measurable and regression is sensibly linear, that no fundamental advance has been made for several years. Boas's[1] first formula is, as pointed out by Pearson,[2] merely another form of the difference method, which has been in use for many years.

Several modifications of a purely technical nature which facilitate calculation or are useful in special cases have been pub-

[1] Boas, F., "Determination of the Coefficient of Correlation," *Science*, N. S., *29*: 823–824. 1909.

[2] Pearson, K., "Determination of the Coefficient of Correlation," *Science*, N. S., *30*: 23–25, 1909.

lished. Pearson[3] has given a new approximate difference method which is serviceable in special cases only. Harris[4] has suggested a novel difference method for exact work with tables. An alternative method of calculating rough moments and product moments, given by Elderton,[5] seems to have attracted little attention, although it has certain advantages for use in adding-machine computations. A product moment method which possesses marked advantages for use with machines which allow of simultaneous multiplication and summation, and which obtains incidentally the data necessary for testing linearity of regression or computing the correlation ratio, η, is now available.[6] In the special cases in which the two characters to be centered in the correlation table are not differentiated, *e. g.*, stature of pairs of brothers, length of *Paramecium*, etc., the tables are ordinarily rendered symmetrical by using each individual once as the x and once as the y member of the pair. This may be done by actually forming the symmetrical table, or by using the simple formula proposed by Jennings.[7] If, as is frequently the case, more than a single pair of individuals are associated, the labor of forming tables becomes very great. Each individual of a family, each organ of an individual, or each individual measured from a particular environment, must then be entered in the table in combination with every other one. Since the number of combinations in each class is $n(n-1)$ and the number of classes must be at least moderately large, the total number of combinations is very great. Thus the data for number of nipples in swine recently published by Parker and Bullard[8] require a table of 34,884 combinations to determine the fraternal correlation for number of nipples. In the case of the *Hydra* data analyzed by Lashley,[9] tables with from one to nearly two hundred thousand

[3] Pearson, K., ''On Further Methods of Determining Correlation,'' Drapers' Company Research Mem., Biom. Ser., IV, Dulan and Co., 1907.

[4] Harris, J. Arthur, ''A Short Method of Calculating the Coefficient of Correlation in the Case of Integral Variates,'' *Biometrika*, 7: 214–218, 1909.

[5] Elderton, W. P., ''An Alternative Method of Calculating the Rough Moments from the Actual Statistics,'' *Biometrika*, 4: 374–378, 1905. Also in his ''Frequency Curves and Correlation.''

[6] Harris, J. Arthur, ''The Arithmetic of the Product Moment Method of Calculating the Coefficient of Correlation,'' Amer. Nat., 44: 693–699, 1910.

[7] Jennings, H. S., ''Computing Correlation in Cases Where Symmetrical Tables are Commonly Used,'' Amer. Nat., 45: 123–128, 1911.

[8] Parker, G. H., and C. Bullard, *Proc. Amer. Acad. Arts and Science*, 49: 399–426, 1913.

[9] Lashley, K. S., *Jour. Exp. Zool.*, 19: 210, 1915.

combinations are given. Methods for the rapid formation of symmetrical tables from which either correlation or contingency coefficients may be calculated[10] and for the formation of condensed tables from which correlation coefficients[11] only may be deduced greatly reduce the necessary labor in such cases. For the testing of linearity of regression in the case of these intra-class and inter-class correlations, tables are essential. The use of such coefficients would, however, be greatly facilitated if calculation could be carried out directly from moments computed from the classes themselves. Harris[12] has given an exhaustive series of formulæ by which this can be accomplished, with examples showing the wide applicability of such coefficients. For example, these formulæ fulfil more adequately the purpose of Boas's second formula (*loc. cit.*).

These intra-class correlation formulæ have been thrown into a form suitable for measuring substratum heterogeneity in experimental cultures.[13]

If the x and y character of a pair are differentiated, spurious values of the correlation coefficient must result from the rendering symmetrical of the correlation surface. Pearson many years ago recognized the difficulty in dealing with groups in which there is orderly differentiation due, for example, to growth.[14] Attention has recently been directed[15] to difficulties arising when differentiation within the class may exist, but it may be difficult or impossible to arrange the individuals by any character outside of themselves to obtain the constants necessary for determining the true correlation from the spurious values deduced

10 Harris, J. Arthur, "On the Formation of Correlation and Contingency Tables when the Number of Combinations is Large," AMER. NAT., *45:* 566–571, 1911.

11 Harris, J. Arthur, "The Formation of Condensed Correlation Tables when the Number of Combinations is Large," AMER. NAT., *46:* 477–486, 1912.

12 Harris, J. Arthur, "On the Calculation of Intra-class and Inter-class Coefficients of Correlation from Class Moments when the Number of Possible Combinations is Large," *Biometrika, 9:* 446–472, 1913.

13 Harris, J. Arthur, "On a Criterion of Substratum Homogeneity or Heterogeneity in Field Experiments," AMER. NAT., *49:* 430–454, 1915.

14 Pearson, K., "On Homotyposis in Homologous but Differentiated Organs," *Proc. Roy. Soc. Lond., 71:* 288–313, 1903.

15 Harris, J. Arthur, "On Spurious Values of Intra-Class Correlation Coefficients Arising from Disorderly Differentiation within the Classes," *Biometrika, 10:* 412–416, 1914.

from the tables. Whether the methods used in such cases by Harris[16] will prove the best available remains to be seen.

Considerable attention has recently been given to the probable error of the correlation coefficient.

If the number of observations upon which r is based is large and if it does not approach too closely either of its limiting values of $+1$ or -1, the use of the formula of Pearson and Filon,

$$E_r = .6745 \frac{1 - r^2}{rn},$$

readily evaluated by the use of the tables of $1 - r^2$ given by Soper[17] used in connection with the x_1, of Miss Gibson's Tables[18] or approximated by the Abac of Heron,[19] is quite legitimate. But when either of these conditions is not realized the value of r found from a single sample will probably not be the true correlation for the population under consideration.

Chemists, agriculturists, physiologists and many others often must necessarily reason from a relatively small number of observations. It is therefore of very real importance that some valid measure of the statistical trustworthiness of such coefficients be known. Some of the problems concerning the probable error of r when it approaches its numerical limits or when the number of cases upon which it is based is small are discussed mathematically by Soper[20] as they have been attacked experimentally by "Student."[21] Further contributions to the subject are those of Fisher[22] and of Pearson,[23] who summarizes the series of studies and gives a table to facilitate the interpretation of correlation coefficients based on small samples. He says:

16 Harris, J. Arthur, "On the Significance of Variety Tests," *Science*, N. S., *36:* 318–320, 1912, and *Biometrika, l. c.*

17 Soper, H. E., In "Tables for Statisticians and Biometricians."

18 *Biometrika, 4:* 385–392, 1906. Also in Pearson's Tables.

19 Heron, D., "An Abac for Determining the Probable Errors of Correlation Coefficients," *Biometrika, 7:* 411, 1910. Also in Pearson's Tables.

20 Soper, H. E., "On the Probable Error of a Correlation Coefficient to a Second Approximation," *Biometrika, 9:* 91–115, 1913.

21 "Student," "Probable Error of a Correlation Coefficient," *Biometrika, 6:* 302–310, 1908.

22 Fisher, R. A., "Frequency Distribution of the Values of the Correlation Coefficient in Samples from an Indefinitely Large Population," *Biometrika, 10:* 507–521, 1915.

23 Pearson, K., "On the Distribution of Small Samples"; Appendix I to papers by "Student" and R. A. Fisher, *Biometrika, 10:* 522–529, 1915.

We think it must be concluded that for samples of 50 the usual theory of the probable error of the standard deviation holds satisfactorily, and that to apply it for the case of $n=25$ would not lead to any error which would be of importance in the majority of statistical problems.

The original papers should be read by those who are dealing with coefficients lying near the limits of the range of correlation, or who must work with small samples. Those who can by extra labor obtain larger series of data should do so, for no knowledge of the theory of the probable error can ever take the place of widened series of data, although it may be essential to the interpretation of constants based of necessity on a limited number of observations.

Both Variates Measurable on a Quantitative Scale; Regression Non-Linear.—For cases in which the rate of change in the y character can not be described by a straight line, the proper measure of interdependence is Pearson's[24] correlation ratio, η. The value of the correlation ratio is two-fold. (a) It furnishes a measure of the interdependence of two variates in cases in which the use of the correlation coefficient is not fully justified. (b) It affords a means of testing, by the use of Blakeman's criterion,[25] for linearity of regression. Thus in deciding between the correlation coefficient and the correlation ratio, the calculation of each of the constants may, in critical cases, be necessary.

A further test of the goodness of fit of regression curves has also been given by Slutsky.[26] This method, which involves the well-known χ^2 of Pearson's test for goodness of fit, should have wide usefulness. An illustration of its application has recently been given by Pearl.[27]

One Variate Describable in Multiple Categories, the other Measurable on a Quantitative Scale.—Such cases are occasionally met with in many fields of work. For example, one may desire to know in fractions of a scale ranging from 0 to 1 the relationship between any describable but not measurable environmental

[24] Pearson, K., "On the General Theory of Skew Correlation and Non-Linear Regression," Drapers' Co. Res. Mem., Biom. Ser., II, Dulan and Co., 1905.

[25] Blakeman, J., "On Tests for Linearity of Regression in Frequency Distributions," *Biometrika, 4:* 332–350, 1905.

[26] Slutsky, E., "On the Criterion of the Goodness of Fit of Regression Lines and on the Best Method of Fitting them to the Data," *Jour. Roy. Stat. Soc., 77:* 78–84, 1914.

[27] Pearl, R., "An Important Contribution to Statistical Theory," AMER. NAT., *48:* 505–507, 1914.

factor and any measurable characteristic of the organisms subjected to its influence. Or in testing the assertions of such writers on criminology as Lombroso and Havelock Ellis against the results of actual measurements of criminals, one may find it desirable to correlate between the kind of crime and any cephalic measurement.

For the analysis of such data the correlation ratio may be of great service.

One Character Alternative, the other Measurable on a Quantitative Scale.—Suppose now that one of the correlation ratio tables of the kind discussed in the foregoing paragraph were reduced, as far as the qualitatively appreciable but not measurable character is concerned, to two classes only, while the measured variate remained as before. Such tables actually occur in practise with great frequency. For example, one may wish to correlate between the form of a dimorphic crustacean and physical measurements. Or it may be desirable to ascertain the correlation between type (tubular or ligulate) of a composite flower and the number of divisions in the corolla. Or one may wish to measure the relationship between type and time required for germination in the seeds of a dimorphic plant species. Or a series of individuals may be classified by the social worker or prison warden as alcoholic and non-alcoholic and the investigator desires to correlate between alcoholism (which is really a graduated character, although classified in the available records into the two alternative classes only) and any physical measurement or the extent of criminality as measured by number of convictions or months spent in prison.

In this reduced form the data can no longer be treated by the correlation ratio method, but must be attached by a recent formula due to Pearson,[28] and known as the Bi-serial correlation coefficient.

Soper[29] has continued his work on the probable error by determining the standard deviation of constants calculated by this formula.

Both Characters Classified in Multiple Categories.—If instead

[28] Pearson, K., "On a New Method of Determining Correlation Between a Measured Character *A*, and a Character *B* of Which Only the Percentage of Cases Wherein *B* Exceeds (or Falls Short of) a Given Intensity is Recorded for Each Grade of *A*," *Biometrika*, 7: 96–105, 1909.

[29] Soper, H. E., "On the Probable Error of the Bi-serial Expression for the Correlation Coefficient," *Biometrika*, 10: 384–390, 1914.

of both characters being measurable on a quantitative scale, or one character recorded in a number of categories and the other measurable on a quantitative scale, *both* characters are not quantitatively measurable, but describable in a number of classes only, neither the correlation coefficient nor the correlation ratio can be used. In such cases, which in practical work are very frequent, Pearson's contingency methods[30] must be used. These have been too long in use to require discussion or illustration here. Certain corrections to be applied will be considered at another time.

The probable error of the contingency coefficient presents considerable difficulty. Those who have to deal with it should consult papers by Blakeman and Pearson[31] and by Pearson.[32]

One Variate Classified in Alternative, the Other in Multiple Categories.—Consider a contingency table reduced to a two-fold grouping for one of the characters, but retaining the multiple division for the other. Such a table is comparable with the condensation of the correlation ratio table discussed above. It must be analyzed by a special method.[33]

The formula has not as yet had extensive practical application. It has been used to determine the relationship between alcoholism as an alternative character and type of crime classed in multiple categories, and between alcoholism in the parent and health of the children. It may prove especially valuable in dealing with the interrelationship of various teratological conditions in morphological work.

Both Characters Classified in Alternative Categories Only.—As the extreme case we may think of a contingency table reduced to a two-fold grouping for each of the characters. This is then the four-fold table for alternative characters, *i. e.*, (A) and (not -A), (B) and (not -B).

In the past, two methods have been chiefly employed for obtaining constants from such tables, Pearson's four-fold correlation coefficient and Yule's coefficient of association.

[30] Pearson, K., "On the Theory of Contingency and its Relation to Association and Normal Correlation," Drapers' Co. Res. Mem., Biom. Ser., I. Dulan & Co., 1904.

[31] Blakeman, John, and K. Pearson, "On the Probable Error of Mean Square Contingency," *Biometrika*, *5:* 191–197, 1906.

[32] Pearson, K., "On the Probable Error of a Coefficient of Mean Square Contingency," *Biometrika*, *10:* 570–573, 1915.

[33] Pearson, K., "On a New Method of Determining Correlation when One Variable is Given in Alternative and the Other in Multiple Categories," *Biometrika*, *7:* 248–257, 1909.

For several years critical workers have realized that very little reliance is to be placed upon Yule's very simple coefficient of association. This coefficient and another measure of correlation "the theoretical value of r" proposed in his "Introduction to the Theory of Statistics" have been discussed by Heron.[34] Pearson and Heron[35] and Pearson[36] have gone into these methods and others proposed by Yule[37] in a masterly way. To discuss this memoir alone would require far more than the space available for this general index of the correlation methods. Their treatment can leave no doubt—if any existed in the minds of those who have tried to use these formulæ in serious statistical work—that except in very special cases all these association and colligation formulæ are likely to work harm rather than to be of service in the hands of the biologist.

This demonstration of the untrustworthiness of the various substitutes for the correlation coefficient practically throws us back upon the old four-fold method of Pearson, and upon another novel method to be discussed in a moment. The difficulty of computation has been one of the greatest obstacles in the way of the more general application of this method and has frequently resulted in the substitution of the less reliable coefficient of association. The necessary labor of calculation has been much reduced by two series of tables by Everitt.[38]

The determination of the probable error of the coefficient of correlation calculated from the four-fold grouping has always been excessively laborious. While four-fold correlations have been calculated in hundreds of cases, the determination of the probable error has been made for less than a hundred of the coefficients. Pearson[39] has now given tables to facilitate the cal-

[34] Heron, D., "The Danger of Certain Formulæ Suggested as Substitutes for the Correlation Coefficient," *Biometrika, 8:* 109–122, 1911.

[35] Pearson, K., and D. Heron, "On Theories of Association," *Biometrika, 9:* 159–315, 1913.

[36] Pearson, K., "Note on the Surface of Constant Association," *Biometrika, 9:* 534–537, 1913.

[37] Yule, G. U., "On the Methods of Measuring Association between Two Variates," *Jour. Roy. Stat. Soc., 75:* 579–641, 1912.

[38] Everitt, P. F., "Tables of the Tetrachoric Functions for Four-fold Correlation Tables," *Biometrika, 7:* 437–451, 1909; "Supplementary Tables for Finding the Correlation Coefficient from Tetrachoric Groupings," *Biometrika, 8:* 385–395, 1912. Also in "Tables for Statisticians and Biometricians."

[39] Pearson, K., "On the Probable Error of a Coefficient of Correlation as

culation of approximate probable errors which are sufficiently exact for all practical purposes.

Finally, the most important recent development in the theory of correlation is probably Pearson's novel method of dealing with variates classed in alternate categories only.[40]

The fundamental conception of this method is exceedingly simple. Given the table,

	A_1	A_2	Totals
B_1..............	a	b	$a+b$
B_2..............	c	d	$c+d$
Totals.........	$a+c$	$b+d$	N

where the large letters represent any alternative (*e. g.*, Mendelian) characteristic of an individual, and the small letters denote the frequency of occurrence of the several possible combinations, it is clear that

$$\frac{a+c}{N},\ \frac{b+d}{N},\ \frac{a+b}{N},\ \frac{c+d}{N}$$

give the independent probabilities of the two pairs of characteristics. The four pertinent products of these ratios give the chances on the assumption of the independence of the two characters A and B, of the four possible combinations. Then if there be no correlation, within the limits of the errors of random sampling

$$a - N\left(\frac{a+c}{N} \times \frac{a+b}{N}\right) = 0,$$

and so on. The squares of the four differences between the observed frequencies, a, b, c, d, and those which would be expected if the two characters were really independent, gives the familiar χ^2 of Pearson's test for goodness of fit. The significance of this test may be determined from Palin Elderton's tables.[41] and this is, in the case in hand, a measure of correlation. It has been

Found from a Four-fold Table,'' *Biometrika, 9:* 22–27, 1913. Also in Tables for Statisticians and Biometricians.

[40] Pearson, K., ''On a Novel Method of Regarding the Association of Two Variates Classed Solely in Alternate Categories,'' Drapers' Co. Res. Mem., Biom. Ser., VII, Dulan and Co., 1912.

[41] Elderton, W. P., ''Tables for Testing the Goodness of Fit of Theory to Observation,'' *Biometrika, 1:* 155–163, 1901. Also reprinted in Pearson's volume of tables.

used as such during the past several years by some of us in practical problems in which we found it impossible to place reliance upon Yule's coefficients and did not feel warranted, because of underlying assumptions, in depending solely upon the classical four-fold method. But it is a measure given in terms utterly incomprehensible to the ordinary mind, which is quite incapable of thinking in millions or in multiples of millions.

What Pearson has done with such brilliancy is to furnish a means in mathematical theory and working tables of passing from the incomprehensible scale of pure probability to the familiar and usable and widely comparable scale of correlation.

As yet it is too soon to be able to state the results of extensive practical application of the new coefficients, but they should have wide usefulness.

Both Characters Classified by Rank in Series.—In some cases, neither measurements nor classification of the individuals dealt with in categories are given in the data, but merely their position or rank in the series.

Rank may be numerically expressed, and the suggestion has been made that the correlation of grades or ranks is a quite legitimate measure of interdependence in such cases. Pearson[42] has, however, pointed out the very real difficulties encountered in such work. Those who are tempted to use these methods should acquaint themselves with the dangers as pointed out in this memoir.

One Variate Given by Rank in Series, the Other Measured on a Quantitative Scale.—Such cases are not likely to occur with great frequency in biological work. Possible instances are those in which one wishes to correlate between position in an intensity of pigmentation series and size or fertility—both quantitatively measurable characters. The formulæ have been given by Pearson.[43]

One Variate Given by Multiple or Broad Categories, the Other by Rank in Series.—Practical applications in biology should be rare. For formulæ see the paper by Pearson just cited.

From the foregoing outline it must be clear that of recent years the conception of correlation has been greatly extended and the possibilities of the practical usefulness of correlation methods

[42] Pearson, K., "On Further Methods of Determining Correlation," Draper's Co. Res. Mem., Biom. Ser., IV, Dulan and Co., 1907.

[43] Pearson, K., "On an Extension of the Method of Correlation by Grades or Ranks," *Biometrika, 10:* 416–418, 1914.

vastly increased by the deduction of formulæ suitable for dealing with data of the most diverse sorts.

The most valuable feature of a summary such as the present may possibly not lie in the fact that it exhibits to biologists the wide array of statistical tools which are now available for dealing with the most diverse sorts of data which they may collect, and shows where directions for their use may be found, but in the suggested warning that the hasty application of the first learned or the most easily calculated formula may lead to constants of little value. Most biologists can use a scalpel or a beaker with great success, but many at least would hesitate to try to handle without special training all the instruments which are to be seen in the surgeon's case or to use all the glassware on the organic chemist's shelves. Each kind of tools require their special training. Notwithstanding popular conceptions to the contrary, this is also true of the biometric tools.

J. ARTHUR HARRIS

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A FURTHER ANALYSIS OF THE HEREDITARY TRANSMISSION OF DEGENERACY AND DEFORMITIES BY THE DESCENDANTS OF ALCOHOLIZED MAMMALS

CHARLES R. STOCKARD and GEORGE PAPANICOLAOU

Department of Anatomy, Cornell University Medical School, New York City

Introduction.
Material and methods.
Direct effects of the alcohol treatment on the animals.
Influence of the treatment on the descendants of alcoholized animals.
The influence of internal and external factors on the quality of the offspring.
Relative conditions of the male and female descendants from paternal and from maternal alcoholized ancestors.
General considerations.
Summary and conclusions.
Literature cited.

Introduction

A little more than two years ago the senior author (Stockard, '13) recorded in this journal experiments which had then been running for three years and seemed to show a definite injury of the germ cells by treating mammals with the fumes of alcohol. This injury of the male germ cells is of such a nature that an alcoholized male guinea pig almost invariably begets defective offspring even when mated with a vigorous normal female. At that time it was also shown that F_1 animals, the offspring of treated parents, though themselves not treated,

had the power to transmit the defective condition to their young, and such F_2 young were equally if not more defective than the immediate offspring of the treated animals.

In 1914 in a short abstract Stockard showed further that the offspring from F_2 individuals were apparently more defective than their parents and were often grossly deformed. One case was recorded of the occurrence of a litter of two F_3 animals, both of which were extremely weak and neurotic, showing a condition suggesting paralysis agitans, and further than this the two animals were typical anophthalmic monsters. The eyes were completely absent, no optic nerve or optic chiasma or visible optic tracts along the tuber cinereum could be found on a careful gross examination of the brain. The two animals were produced by parents (F_2) that had never been treated with alcohol, the four grandparents (F_1) had also not been treated, while the *three great grandfathers* had been alcoholized and the three great grandmothers were normal untreated individuals.

Defective eyes and absence of one eye or both eyes have been frequently met with in the experiment, as well as the peculiar nervous condition, and these symptoms are to be considered indicative of the injury or change induced in the male germ cells by the experimental treatment, which in the above case was transmitted through three generations. No question could remain as to the action on the germ cells, as only male ancestors had been treated; every female of the line was an untreated animal.

This abstract called attention to the fact that there was a tendency for the results to differ in subsequent generations from treated males as compared with the descendants of treated females—not enough data were then present to offer any explanation of these differences and a consideration of them will be undertaken in the present paper.

At that stage of the experiment it was also difficult to offer an exact analysis of the mode of transmission of

the defects and the type of injury induced by the alcohol treatment, since the total numbers were not large and the F_2 animals had only a few matings, while further generations had not become available for breeding.

The same experiments have now been continued for more than five years and a number of animals have been used, over 700, which cover the behavior of four generations and supply data of sufficient extent to allow a more thorough analytical consideration of the heredity problem concerned.

Experiments of this nature on mammals are fraught with many difficulties, slowness of breeding, small size of litters, difficulty of handling, etc. Yet such material offers one very great advantage in that the quality of the offspring and generations studied is of such a complex that one is enabled to detect indications of rather slight injuries or changes in the material carriers of heredity which would not become evident on lower forms with less diversity in their methods of behavior and structural appearance. In other words, we take it that such conditions as are spoken of as racial degeneracy in man and mammals are often very difficult or even at times impossible to detect in lower forms.

These conditions are for many reasons thought to be inherited. If so their inheritance must be due to a pathological condition of the material carriers of heredity, the chromosomes, or what not, since they are not normal states and, like diseases, are constantly arising in normal families on account of one or another form of intoxication. Is it possible then to produce such a racial degeneracy artificially by treating only one generation of the animals and by so doing observe a pathological behavior of the carriers of heredity? Arguing from analogy there must be pathological heredity due to diseased or altered chromosomes in the germ cells just as truly as there is a known pathological behavior of every other organ and tissue of the animal body.

It becomes then a problem to study the possible meth-

ods of modifying the chromosomes or carriers of the inherited qualities of organisms in order to further analyze their normal physiological behavior; in the same way that experimental embryology has been able to supply so many valuable clues to the normal processes of development.

In the following pages we believe the facts indicate that individual guinea pigs are now living in this experiment that have had the carriers of hereditary qualities, the chromatin, of their germ cells injured for a longer time than four years. And during this time they have given rise to offspring of more or less degenerate or deformed type, and in some cases these offspring have passed this modified chromatin on through three generations, all of which contain pathological chromatin and show somatic defects and deformities as an index of their tainted chromatic ancestry. Modified chromatin has been living in the experiment for more than four years in five different generations of animals as a result of the treatment on the one original, P_1, parent generation.

We have tried to regulate every controllable source of error and there can be no doubt that the conditions are brought about in the way described. Could the degeneracy which is so pronounced have previously existed in the stock? This question has been controlled in the first place by the use of two entirely different stocks from different sources and obtained one and one half years apart, the first in the fall of 1910 and the other in the early winter of 1912. The responses of the two stocks to the experimental treatment have been identical. As a second method of control every animal has been tested by one or more normal matings before being introduced into the experiment, and only those giving normally strong offspring have been used. A further crucial control is the constant mating of normal untreated animals from both stocks under identical cage conditions with the experimental individuals. These animals continue to breed normally until very old, when they gradually become

sterile. But none have ever given rise to a defective or deformed individual, and the rate of mortality of the young indicates the average healthy condition found in normal guinea-pig breeding. There is a striking contrast between the records of these normal young and the mortality record, the frequency of easily recognized nervous symptoms of degeneracy, and the prevalence of gross deformities in the experimental races.

The external as well as internal factors are to be considered not only in individual or embryonic development, but also in heredity. And the present experiments now demonstrate for mammals that either the spermatozoon or the ovum may be experimentally injured or modified in such a manner as not only to give rise to (abnormal) subnormal development in the resulting embryo, but the effects of the injury may be transmitted from generation to generation, until an affected line actually fades out through degeneracy and sterility as a result of the transmitted condition.

Material and Methods

The animals used in the experiments have been ordinary vigorous guinea pigs of large size, particular care being taken to select animals less than one year old to begin with and good breeders.

At the beginning of the experiments alcohol was given along with the food, but the animals ate less and the food usually disagreed with them. It was then administered in diluted form by a stomach tube; this method was even more unsuccessful, disturbing digestion and seeming to upset the animals considerably. It is certain that alcohol given to animals through the stomach deranges their appetite and digestion to such an extent that the experimenter is unable to determine whether the resulting effects are due to the alcohol, as such, or to the generally deranged metabolism of the animal. When given in drinking water they take little or none of the water and the treatment is insufficient. For these reasons an inha-

lation method of treatment was resorted to early in the study, and, as far as experience goes, it has no serious disadvantages and does not complicate the conditions of the experiment.

This method may be merely described in brief for the convenience of the reader, since it has been fully recorded with illustrations of the fume tanks in previous publications. A fume tank of copper is made of sufficient size to supply breathing space for four or five guinea pigs at one time. The tank has four outlets, so that a definite amount of fumes may be passed through in a given time and the ventilation controlled. In this way each animal could be given a definite measured dose. The individuals, however, differ so much in their resistance to the treatment that it has been found better to treat all to about the same degree of intoxication. Such a physiological index is more reliable, since every animal may be affected to the same degree each day. For this purpose the animals are placed in the fume tank on a wire screen, and absorbent cotton soaked with alcohol is placed beneath the screen, so that they inhale the alcohol fumes arising from the cotton to saturate the atmosphere of the tank.

Ether was given in a similar manner. The animals are much more readily overcome by these fumes and must be carefully watched while inhaling even the most dilute doses.

To avoid handling the females during pregnancy, special treating cages are devised. An ordinary box-run with a covered nest in which the animal lives is connected by a drop-door with a metal-lined tank, having a similar screen arrangement, etc., to that of the general treatment tank. The pregnant animal may be driven daily into the tank and thus treated with alcohol fumes throughout her pregnancy without being handled in any way that might disturb the developing fetus.

Particular care is necessary in mating the animals in such an experiment, as the females are often slow to con-

ceive and some of the F_2 and F_3 individuals of both sexes are not very prolific and in many cases are almost or quite sterile. Each female is kept in a separate run and the male is placed in with her just before the time of the expected heat period, ovulation, and he remains in her cage for from two to three weeks so as to be present at the second ovulation, provided the female was not made pregnant by the first mating. The ovarian cycle of the guinea pig as worked out by L. Loeb seems to correspond closely to what is found in mating experiences.

After mating, the male is removed from the cage and the female remains alone until the young are born. These are left with the mother for about fifteen days, then separated, and the female mated again. In this way the normal females may sometimes give as many as four litters per year, but the experimental animals breed much slower and it is difficult to get even three litters per year.

Direct Effects of the Alcohol Treatment on the Animals

Several of the guinea pigs have now been treated with the fumes of alcohol almost to the point of intoxication for six days per week for a period of five years. This is a considerable space in the life of a guinea pig, which probably would not often extend beyond six or seven years.

The animals are affected by the alcohol fumes in various ways; some of them are stupefied and become drowsy, while others become stimulated and excited and sometimes even vicious, constantly fighting and biting at the other animals in the fume tank. The fumes inhaled into the lungs pass directly into the circulation, so that the animals show signs of intoxication very soon after being put into the tank, yet the intake of alcohol is so gradual that they may remain for one hour or more without becoming totally anesthetized.

The mucosa of the respiratory tract is considerably

irritated during the early stages of the treatment, but develops a resistance so that later little effect can be noticed. The cornea of the eye is greatly irritated, often becoming milky white and opaque during the first few months. In some cases this later clears and the animal is again able to see, though some of the animals treated for several years have remained entirely blind. The general condition of the animals under the fume treatment is very good. They all continue to grow if the treatment is begun before reaching their full size, and become fat and vigorous, taking plenty of food and behaving in a typically normal manner.

Some of the treated animals have died and others have been killed at different times during the progress of the experiment and their organs and tissues examined carefully and then studied microscopically. All have seemed practically normal. Tissues from several animals treated as long as three years have been examined and the heart, stomach, lungs, kidneys, and other organs present no noticeable conditions that might not be found in normal individuals. Alcoholized animals are usually fat, but there is no fatty accumulation in the parenchyma of any of the organs.

Several of the animals, both males and females, have been partially castrated during the experiments and the ovaries and testes have been found to be in a healthy condition, though certain possible changes may be present which are now being closely studied cytologically and experimentally.

The treated animals are, therefore, little changed or injured so far as their behavior and structure goes. Nevertheless, the effects of the treatment are most emphatically shown by the type of offspring to which the alcoholized individuals give rise, whether they be mated together or with normal individuals. The further significance of the nature of the effects is indicated by the quality of the subsequent generations descended from such an ancestry.

Influence of the Treatment on the Descendants of Alcoholized Animals

It may be well in the first place to consider the results of the experiments from a general standpoint and then to undertake an analysis of the reactions and conditions presented in the several generations and from the several lineal combinations. The records of the matings of the alcoholized animals in various pairs, the control or normal matings, and the matings of the F_1 and F_2 generations, the children and grandchildren of the alcoholized individuals are summarized in the general Table I. This table gives a record of all the matings of the kinds indicated up to July 1, 1915. A similar table was published two years ago, when the number of animals considered was much smaller and the actual indications from the results were less certain than now. On comparing this table with the former one, however, it will be seen that the continuation of the experiments has fully substantiated the results as previously recorded. The table now shows the records of 571 matings which produced 682 full-term young and 189 early abortions or negative results. These numbers are now of considerable magnitude in spite of the fact that the experiment is conducted on mammals which produce only small litters and breed slowly as compared with lower animal forms.

In the first horizontal line the record of pairing alcoholized male guinea pigs with normal females is given. This combination could only produce defective or subnormal young as a result of the injured male germ cells, since the ova are normal and develop in a normal untreated mother. This then is the definite test of the influence of the alcohol treatment on the germ cells.

Ninety such matings have in 37 cases given negative results; that is, failures to conceive, or early abortions. Thus 41 per cent. of the matings of such males were nonproductive, while less than 25 per cent. of normal matings under the same breeding conditions failed to produce full-term litters. Ten stillborn litters, each consisting of

two young, twenty stillborn young, resulted from the 90 matings. While the 90 control matings gave only two stillborn litters, and in both cases these were unusually large litters of four individuals each, and they were probably dead on account of the fact that the mother could not give normal birth to so many offspring. The stillborn litters by the alcoholized fathers were all ordinary-size litters of two young. Thus, while 11 per cent. of the matings of alcoholized males resulted in stillborn litters, only 2 per cent. stillborn litters occurred from normal matings. Forty-three living litters were produced or a little less than 48 per cent. of the matings gave full-term living young, while 73 per cent. of the normal matings give living litters of young.

The 43 litters from alcoholic fathers contained in all 82 young, and 35, or almost 43 per cent., of these died soon after birth, while 66 similar litters from the control lost only 19 young, or 16 per cent., out of 118 individuals. Finally, then, from the 90 matings of alcoholic males with normal mates only 43 full-term litters resulted, consisting in all of 102 young; 55 of these, or 54 per cent., died at birth or soon after, and only 47 individuals, or 46 per cent., survived. Only about half as good record as the 78.5 per cent. surviving young from the matings of normal animals. Almost all of the offspring were very excitable, nervous animals and three of them showed gross deformities of the eyes, while no such conditions were found among any of the offspring of normal animals bred under identical conditions.

These records leave no doubt that the alcoholized male guinea pig is injured in such a way as to induce a decidedly bad effect upon the quality and mortality of his offspring when compared with the records from normal animals.

The second horizontal line of Table I shows the results obtained when alcoholized female guinea pigs are paired with normal males. In this case there is a double chance to injure the offspring. First through the influence of

the treatment on the oocytes or the unfertilized ovarian egg, a direct effect on the germ cells comparable to the injury of the germ cells in the case of the treated males considered above. While in the second place, the developing embryo in the uterus of an alcoholized female may be directly affected by the strange substances contained in the blood and body fluids of the mother. Thus a defective individual may be produced as a result of development in an unfavorable environment or as a result of being derived from an injured or defective egg cell.

Thirty-three matings of alcoholized females with normal males have in seven cases, 21 per cent., given negative results or early abortions; this compares very favorably with the records of the control animals. Four stillborn litters consisting of three individuals each were produced. This is a record of 12 per cent. stillborn litters against only 2 per cent. from normal matings. The alcoholized females gave birth to 22 living litters containing 44 young, and 23, or 52 per cent., of these died, only 48 per cent. surviving against 84 per cent. survivals among the young of similar control litters. The records of the matings of alcoholized females compare very unfavorably with the record of the control matings. Yet the behavior of the alcoholized females is very little, if any, worse than the records shown by the alcoholized males in spite of the double chance the female has to injure her young.

The third horizontal line of the table indicates the results obtained when alcoholized males are paired with alcoholized females. Here there is every chance for the treatment to show its effect. The percentage of early abortions or negative results is very high, about 50 per cent. more than double that of the control matings. Ten per cent. of the matings produced stillborn litters each consisting of two young. Only 17 living litters were born out of 41 matings, about 41 per cent., against 73 per cent. living litters from 90 control matings. The 17 living lit-

ters contained only 26 young, and 12 of these, or 46 per cent., died soon after birth, while but 16 per cent., or one third as many, of the control offspring died out of a total of 118 individuals. The data from the double alcoholic matings is, therefore, extremely bad in the light of normal matings from the same animal stocks bred under exactly the same cage and food conditions.

The fourth horizontal line summarizes the records of all the matings of directly alcoholized animals. In all 164 such matings have been made; 64 of these, or almost 40 per cent., gave negative results or early abortions. Eighteen stillborn litters occurred, consisting of 40 individuals against only two questionable stillborn litters from 90 control matings. Eighty-two, or only 61 per cent., living litters were born, consisting of 152 individuals, 82, or 54 per cent., of which survived and 70, or 46 per cent., died soon after birth; in all 110 full-term young died, while only 82, or 42 per cent., of the total 192 full-term young resulting from the 164 alcoholic matings survived. On the other hand, out of a total of 126 full-term young from only 90 control matings, 99, or 78.5 per cent., survived. The control matings were far more prolific than those of the alcoholized animals and the condition of the young as indicated by the mortality record was far superior to that of the alcoholic offspring.

The fifth line records the outcome of 90 control matings which have been scattered through the entire progress of the experiment under exactly the same conditions and from the same animal stocks as the experimental matings. Eighty-four per cent. of the young in the 66 living litters resulting from the matings of normal animals have survived and all are strong, healthy individuals; in not one instance do they show an indication of nervous degeneracy or any type of recognizable structural deformity, while such degeneracy as well as deformities are extremely prevalent among the offspring and descendants of the alcoholized animals. One other point to be mentioned in considering the records of the

TABLE I

EFFECTS OF ALCOHOL ON THE DESCENDANTS OF TREATED ANIMALS

Condition of the Animals	Number of Matings	Negative Result of Early Abortion	Stillborn Litters	Number of Stillborn Young	Living Litters	Young Dying Soon After Birth	Total Dead	Surviving Young
Alcoholic ♂ ×norm. ♀	90	37	10	20	43	35, 1 e.e.	55	47, 2 o.e.
Norm. ♂ ×alcoholic ♀	33	7	4	12	22	23	35	21
Alcoholic ♂ ×alcoholic ♀	41	20	4	8	17	12	20	14
Summary....................	164	64	18	40	82	70	110	82
Control norm. ♂ ×norm. ♀	90	22	2	8	66	19	27	99
♀ treated during pregnancy.......	4	0	0	0	4	1	1	7
Second generation ×norm..........	46	10	3	8, 6 e.e.	33	29, 2 par.	37	25, 3 e.e.
Second gener. ×alcoholic..........	53	16	8	17, 1 d.e.	29	22, 3 d.e.	39	28
Second gener. ×second gener.......	95	29	7	16	59	43, 2 par., 6 d.e.	59	52, 3 d.e., 1 one e., 1 eyeless
Third gener. ×third gener.........	48	20	7	14, 1 d. legs	21	19, 1 par., 6 d.e., 2 eyeless	33	13
Third gener. ×second gener........	33	15	4	8	14	16, 1 par., 1 e.e.	24	7
Third gener. ×normal.............	17	3	4	8	10	5	13	7
Third gener. ×alcoholic	3	1	0	0	2	2	2	1
Second, third gener. ×second, third gener.......................	18	9	2	6	7	6	12	4

control matings is the fact that from 90 matings only two stillborn litters were produced and, as mentioned above, both of these litters were of so large a size that the mothers seemed unable to successfully deliver them and one of the mothers failed to recover from the process and died a few days later. These two cases make the control records appear worse than they actually should, but in spite of this the control matings have given data equally as good as those generally obtained by careful breeding experiments with vigorous normal stocks. The stock in these experiments is unquestionably good, as the control matings very readily show.

Four normal females were mated and then treated with alcohol throughout their periods of pregnancy and, as the sixth horizontal line of the table indicates, such a treatment was not at all injurious in these particular cases. It actually happened that some of these young were unusually vigorous. The numbers are very small, but this is a direct test, and if such a treatment were really decidedly effective in its action on the embryo or fetus *in utero* these eight young animals should have at least shown some response. It is very possible that after the treatment has been continued for a long time, a year or more, that the mother then presents a uterine environment unfavorable for normal development, since the offspring of such individuals are almost always subnormal. In these cases, however, the inferior quality of the offspring may be due to the action of the alcoholic treatment on the ovarian germ cells rather than the direct environmental effect on the developing embryo or fetus, there is no way at such a stage to separate the two possible effects.

The next three horizontal lines, seventh, eighth and ninth, give the data resulting from the matings in various combinations of the F_1 animals, that is, offspring from alcoholic parentage, but which are not themselves treated with alcohol. The records of these non-treated F_1 individuals are most instructive for an understanding of the actual influences of the alcoholic treatments.

When such F_1 animals are paired with normal individuals the seventh line shows that almost 22 per cent. of the matings failed, which is not a bad record. The proportion of stillborn litters, however, from the F_1 by normal combination was three times as great as from normal matings and 75 per cent. of the stillborn young produced showed gross defects of the eyes, having opaque lenses or typical cataract conditions, while not one of 126 young from normal matings has shown this or any other noticeably abnormal structure. Thirty-three living litters were produced containing in all 54 individuals, 29, or 54 per cent., of which died soon after birth, while 25 survived. Two of those dying soon after birth were paralyzed and unable to walk, while three of the 25 survivors have defective opaque eyes, cases similar to that illustrated by Fig. 1, and many show different nervous symptoms. Thus of 62 full-term young produced by F_1 animals with normal mates, only 25, or 40 per cent., survived for more than a short time after birth, and 12 per cent. of these have gross defects and more than half of them are nervous, excitable individuals, which when mated with normal animals or in any other combination always give very poor quality offspring, if any at all.

The eighth line shows the records of 53 matings between F_1 animals and alcoholics. This combination again gives data comparing most unfavorably with the control and in some ways even worse than the records of matings between two alcoholic animals. Fifteen per cent. of such matings produced stillborn litters! Only one combination gives a worse record of stillborn that is, matings among F_2 animals. Almost half of the young in the living litters died and here again some were deformed. Deformities are strikingly more abundant among the offspring from F_1 and F_2 parents than from the directly alcoholized animals.

The record of 95 *inter se* matings of F_1 animals is shown in the ninth line. Thirty per cent. of such matings gave negative results or early abortions, over 7 per

FIG. 1, A and B. 271 F_2 ♀ (one in litter) (AA)(AN). Both paternal grandparents and the maternal grandfather were alcoholic, no inbreeding. The right eye is smaller than the left and has been entirely opaque since birth. This animal, almost two years old and vigorous, is entirely sterile.

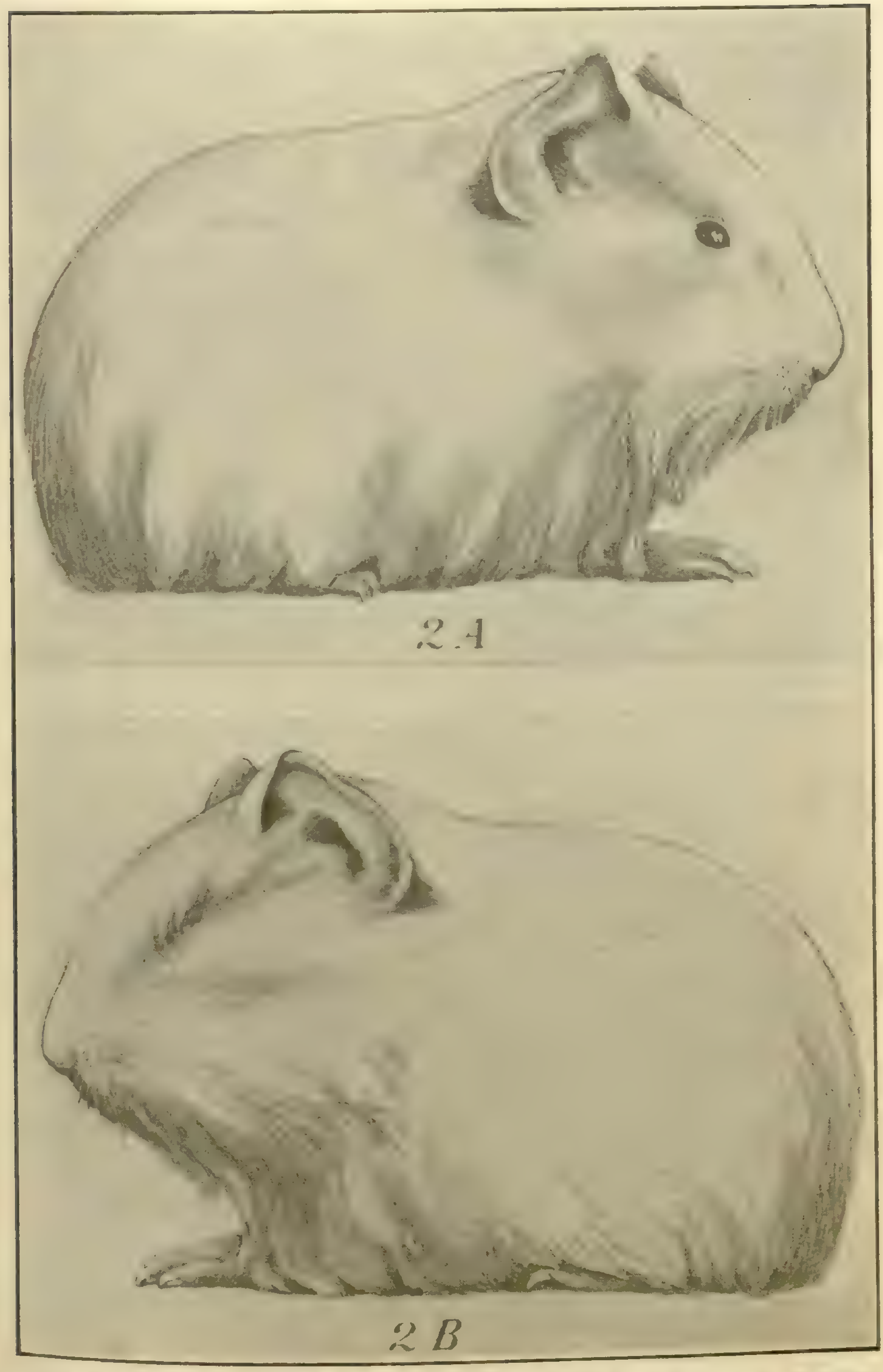

FIG. 2. *A* and *B*. 307 F_2 ♀ (one in litter). Inbred, from brother and sister offspring of an alcoholic male (AN) (AN). The eye of one side normal, the other eye ball apparently absent on living examination. A typical monster monophthalmicum asymmetricum. This animal now 21 months old is completely sterile.

cent. stillborn litters and 62 per cent. living litters. Little less than half of the living young died soon after birth, in all 43, nine of which, or more than one in five, 21 per cent., were paralyzed or deformed; the figures in Plates I and II illustrate the paralytic conditions. Fifty-two of the offspring survived, three with deformed eyes, one with one eyeball completely absent, monster monophthalmicum asymmetricum (Fig. 2, 307 ♀), and almost all of the 52 are very nervous, excitable animals which when bred give rise to deformed or highly degenerate offspring.

The offspring from the F_1 animals mated in any combination are generally far below the normal in power to survive and in quality of structure. When compared with the offspring from directly alcoholized parents, the offspring from the F_1 combinations show an equally bad mortality record and a very much higher proportion of paralyzed and deformed individuals. The 95 matings *inter se* of F_1 animals demonstrate conclusively that such individuals carry defective or abnormal germ cells which give rise to defective developmental products. These degenerate F_2 offspring owe their subnormal condition to the effects of the action of the alcohol treatment upon the germ cells of their grandparents which have been transmitted to them through their parents. In other words, the carriers of hereditary qualities have been modified in the first parental generation, and the effects of this modification are expressed in their offspring F_1, and also in their grandchildren, the F_2 generation.

The next line of the table, the tenth, indicates further how the effects of the original modification are transmitted to the great grandchildren or through three generations since the injury. Forty-eight *inter se* matings of F_2 animals gave the results here shown. Almost 42 per cent. of the matings gave negative results or early abortions, the poorest record in this respect shown in the entire table. About 15 per cent. of such matings gave stillborn litters, 7 in 48 matings, which is remarkably high when compared with any of the above combinations.

The hind legs of one of the stillborn young were deformed in the peculiar manner illustrated in Figs. 4 and 5.

Twenty-one living litters were produced, containing in all 32 young; 19 of these, almost 60 per cent., died soon

FIG. 3. F_3. Two in litter, both same condition, three normal great-grandmothers and three alcoholic great grandfathers. The parents were single first cousins. Both animals completely eyeless, also with paralysis agitans, one died the second and the other the third day after birth, typical anophthalmia. One brain no indication of optic nerve, the other slight processes.

after birth, and only 13 survived. One of the 19 that died was paralyzed and unable to stand, while 8 of them, a strikingly high proportion, were grossly deformed. Six

had one or both eyes deformed (Figs. 1 and 2), and two were anophthalmic monsters, being completely without eyeballs, optic nerves, optic chiasma or any gross signs of optic tracts (Fig. 3). The brains are now being studied in sections. Figs. 1 to 3 illustrate animals showing the different eye conditions—asymmetrical eyes, monstrum monophthalmicum, and anophthalmic monsters. Figs. 5 and 6 of Plate III illustrate the brains of a normal and an anophthalmic specimen for a comparison of the condition of the optic nerves, etc.

Forty-six full-term young were produced by the F_2 matings, but only 13 of these, or just 28 per cent., were able to survive, while about three times this proportion, or 78.5 per cent. of the full-term young from control matings, survived as vigorous healthy individuals. The 13 living F_3 animals are all rather weak and degenerate and almost completely sterile according to a considerable number of careful matings with strong, fertile guinea pigs. The alcoholic race seems at this stage of the experiment about to fade out in the fourth generation, while normal control lines from the same original stocks have passed far beyond this generation, continuing to breed normally and showing no signs of degeneracy, and never in any case giving rise to a grossly deformed animal.

The eleventh line indicates again the very decided effects transmitted by the descendants of animals which had suffered a modification of their germ plasm by the alcoholization of their tissues. In 33 cases F_1 and F_2 animals were paired together. Fifteen of these matings gave negative results or early abortions, while about 12 per cent. of the matings resulted in stillborn litters of two young each. Only 14 living litters were produced by the 33 matings; these contained in all 23 young, only 7 of which survived. Thus from a total of 31 full-term young only 7, or about 22 per cent., were capable of surviving. All of these young animals are nervous and weak and several offspring from these combinations were deformed.

When F_2 animals are mated with normal individuals

the results are very little if any improved over the two above combinations. Seventeen such matings gave only three failures or early abortions, but a high proportion, 23 per cent., of stillborn litters arose, while 10 living litters, consisting of only 12 individuals, were born. In all 20 full-term young were born and only about one in three of them survived. In this experiment, although one mate was a normal animal, the F_2 mate carried germ cells of so inferior a quality that the output of the combination, admitting the numbers are small, leaves no doubt of the transmission, *through three generations,* of defective conditions induced by alcoholizing the great grandparents of the offspring on only one side of the family, or in only one of the parental lines.

The last line of the table gives the records of mixed combinations of F_1 and F_2 individuals, and here the data are closely similar to those obtained from other combinations of these animals; only about 25 per cent. of the full-term young born are capable of surviving, while 78.5 per cent. of the control young are living.

Briefly, then, 571 matings tabulated in Table I, the records to July 1, 1915, have given rise to 682 full-term young, as well as a large number of premature abortions. A careful study of all these young animals extending over a period of five years has afforded data which convincingly show that the treatment of either the male or the female guinea pig with fumes of alcohol affects the quality of the offspring to which these animals give rise even when paired with normal mates. And further, the changed quality of the offspring is subsequently transmitted through succeeding generations with even more severe marks of degeneration and deformity than those exhibited by the offspring of the directly treated animals.

Other combinations and back crosses are now in progress which are fully in line with the above, but which have not yet afforded sufficient analytical data to record.

The defects caused by the alcohol treatment seem to be largely confined to the central nervous system and organs

of special sense. Paralysis agitans is very common among the F_1, F_2 and F_3 animals. Paralyzed limbs are often observed, the animals being unable to stand or walk (Plates I and II). The eye is also a peculiarly sensitive indicator and presents in the various descendants of alcoholized individuals all degrees of degeneration—

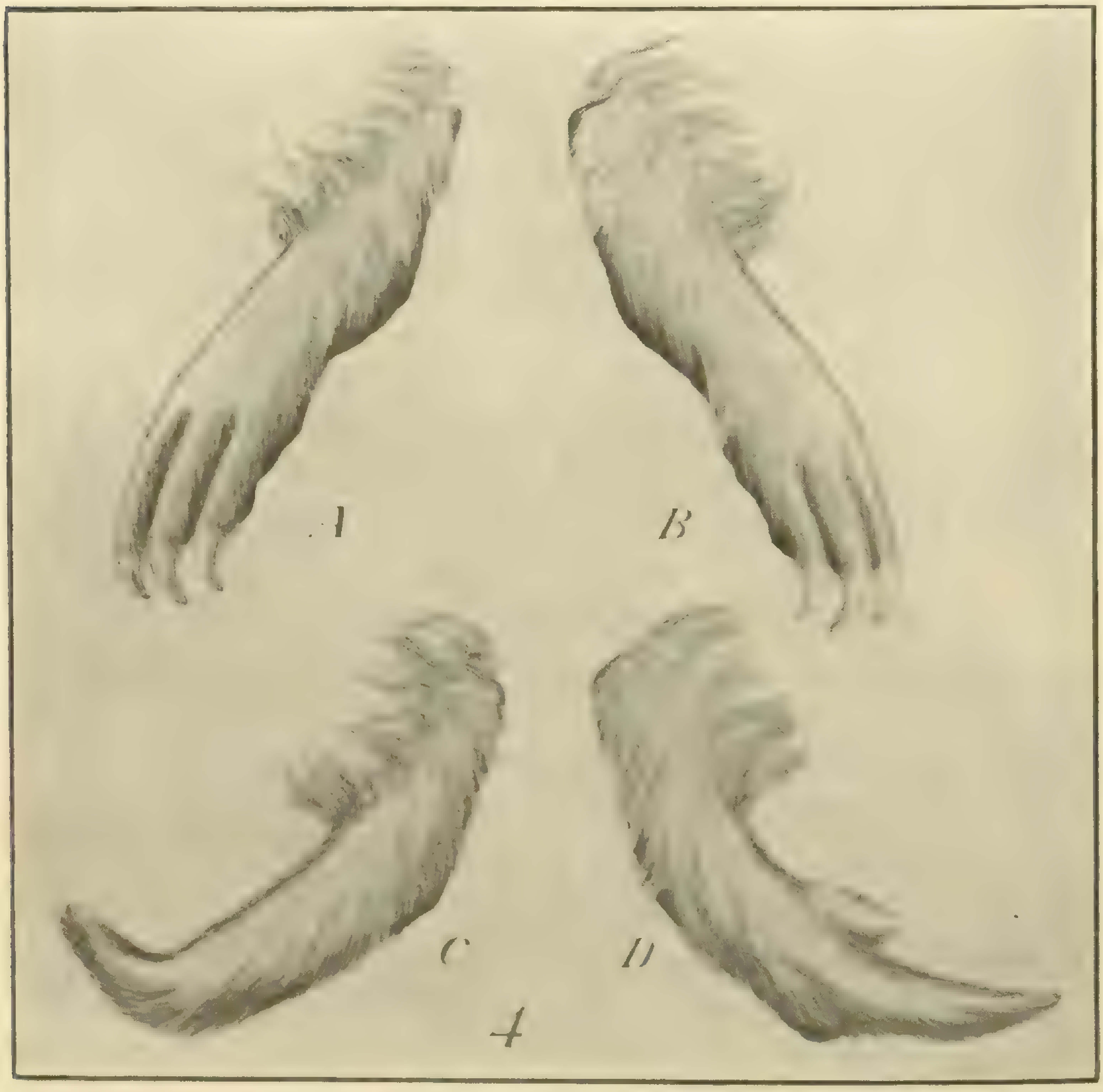

FIG. 4. Hind feet of No. 488 $F_{2,3}$ ♀. All great grandparents were alcoholic as well as the maternal grandparents. Inbred from mother by son. This animal was one of a litter of two stillborn. The left hind foot, *C*, had only one toe and the right, *D*, one toe and a stump. *A* and *B*, normal right and left hind feet.

opaque cornea, cataract or opaque lenses, small defective eyes, complete absence of one eye and finally complete absence of both eyeballs—anophthalmic monsters. In the latter case the extrinsic eye muscles, the third, fourth and sixth nerves, the lachrymal glands and other struc-

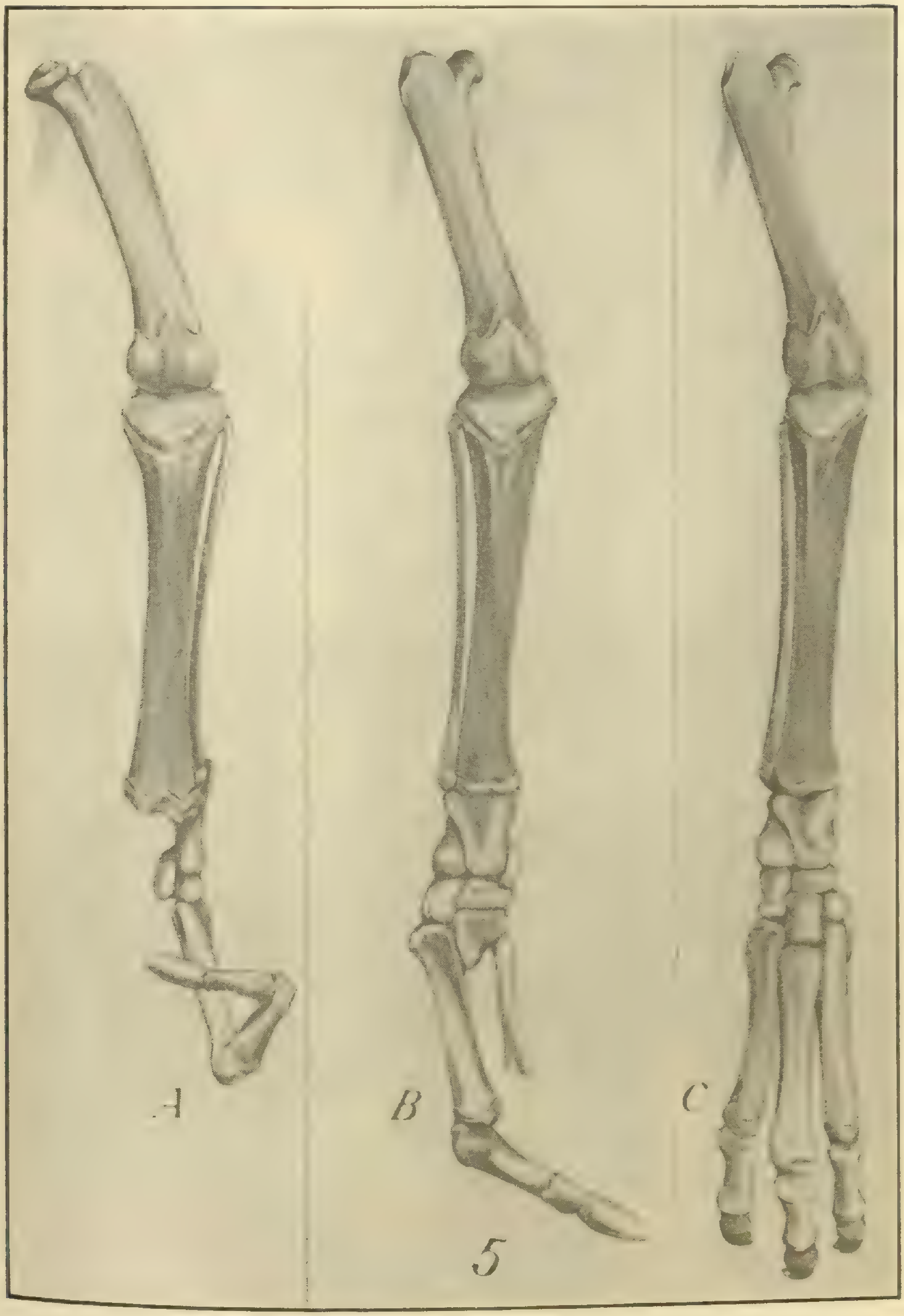

FIG. 5. The skeleton of the two limbs from Fig. 4 shows the left foot, *A*, to have only one metatarsal and toe, the third, and three tarsal bones. The right foot, *B*, has the third toe also and the first metatarsal with the tarsus almost complete. *C* shows the normal skeleton of a right hind limb with the three toes and seven tarsal bones.

tures of the orbit are present, though the eyeball is completely wanting.

Not only are the above congenital eye defects present, but in several instances members of the alcoholic lines have become blind during the first year or year and a half after birth, whereas in our control this has never occurred.

The several illustrations referred to above show specimens exhibiting these various defects. Figs. 1, 2 and 4 of Plates I and II are photographs of animals of indicated lineage which show paralytic conditions. Figs. 4 and 5 illustrate defective extremities. Figs. 1 to 3 show various degrees of defective eyes and absence of eyeball.

It is peculiarly interesting to find these particular eye conditions exhibited by the descendants of alcoholized animals, since, as Stockard ('10) has previously shown, closely similar eye conditions are obtained in great numbers by directly treating the eggs of fish with solutions of alcohol; and like conditions were also obtained, though not so consistently, by treating hens' eggs ('14) with alcohol fumes either before or during incubation.

The table just considered gives only a general idea of the experiment and is in no way analytical. We shall now attempt to analyze these data in such a manner as to determine the influence of internal factors, as, for example, inbreeding on the results. The influence of the size of the litter on the quality of the offspring. The behavior of F_1 and F_2 individuals derived from different lines, and whether there is a difference in the effects on male and female animals, and the manner of transmission of these effects.

(To be continued.)

FECUNDITY IN THE DOMESTIC FOWL AND THE SELECTION PROBLEM[1]

DR. RAYMOND PEARL

I

In the December number of the AMERICAN NATURALIST Professor W. E. Castle[2] directs a vigorous attack against the present writer's work on fecundity. Any one reading Professor Castle's article could scarcely fail, I think, to carry away the impression that the whole of the writer's studies of the past eight years on fecundity in the domestic fowl are to be regarded as essentially valueless. I assume that it was not the intention to convey this impression. The fact, however, appears to be as here stated. With such a conclusion I can scarcely be expected to agree. I shall therefore attempt, in the following pages, in the first place, to call attention to some points regarding my own work which Professor Castle appears to have overlooked, and which seem calculated to give it at least some slight degree of significance, and in the second place, to set forth very briefly my reasons for venturing, in the present state of knowledge, to hold a different opinion from his in regard to some phases of the selection problem.

II

The general plan of Professor Castle's paper appears to be to make a comparison between his selection experiments with rats, and my selection experiments with poultry, to the very great disadvantage of the latter. To this general comparison no general comment on my part can be made, except assent to Castle's conclusion that his

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 94.

[2] Castle, W. E., "Some Experiments in Mass Selection," AMER. NATURALIST, Vol. XLIX, pp. 713–737, 1915.

work on the selection problem is vastly superior to my own. Since the subject of such comparison has been opened it gives me great pleasure to pay tribute, in all sincerity, to Professor Castle's splendid series of experiments on selection in rats. In respect of the numbers of animals involved and their superior adaptability for such an experiment, his work with rats altogether transcends anything which has been done with fowls. These selection experiments constitute an achievement of which their author may well be proud. I have ventured to disagree with Professor Castle's *interpretation* of the results for reasons which will presently be stated. But this difference of opinion, I would most strongly emphasize, concerns *only* the interpretation. We are at one in our high admiration of the factual basis afforded by the rat experiments.

III

Granting all this, however, it seems to me that possibly the case against my studies of fecundity *in toto* is not quite so bad as Castle makes it out to be. Let us examine his points *seriatim*. In the first place the strictures upon the character egg production on p. 714 seem to me to overdo the matter a bit. It is of course true that it is a character confined in its expression to one sex, though that it is also a character which is transmitted by the other sex even Castle somewhat grudgingly admits (p. 715). It also is a character which comes to expression only in the adult. Of this Castle makes a great point throughout his paper, emphasizing that this means that only a small proportion of all offspring born can take part in selection experiments. From the standpoint of methodology this point has nothing like the significance which Castle attributes to it, for the very simple reason that in *all* breeding experiments, his own included, there is a vast amount of random sampling between the population of parental genes and the population of offspring somata. When Professor Castle breeds a pair of rats only a very few

sperm and ova out of the vast hordes the parents produce take part in the production of the resulting litter. He operates, of course, upon the basis that those germ cells which *do* take part in the formation of the litter constitute a *random sample* of the whole population. When I put pullets into the house to test their egg production I operate on precisely the same basis, viz., that I have a random sample of the family from which they are taken. As a matter of fact, I have been at great pains to ensure that the sampling should be random. In all of my studies on the inheritance of fecundity I have regarded this as a point of paramount importance, and have never made use (except occasionally for confirmation of points already made out on other material) of families in which I had not either tested *all* the daughters as to egg production or a sufficiently large random sample to be fairly representative of the family. Further I have repeatedly made careful *ad hoc* investigations of the adequacy and randomness of my sampling.

Castle's next point is, as the matter stands, apparently well taken. He quotes (p. 715) a statement which I somewhat rashly made to the effect "that phænotypic variation of the character fecundity in fowls, markedly transcends, in extent and degree, genotypic variation." Professor Castle's treatment of this statement is perfectly legitimate. If it were true, as stated, it would admit of being turned around as it is in Castle's next sentence, and then it surely would be silly to talk about either selection for this character or about its Mendelian inheritance. What I should have said when I wrote that unfortunate sentence, but did not, was that phænotypic variation *may* transcend genotypic in fecundity, not that it always or regularly does. Because it may I wanted to point out the need for great care in respect of environmental conditions in interpreting results with this character. The real point is this: Long experience in working with winter egg production in poultry has convinced me that under properly controlled environmental conditions this character is as

definitely and regularly controlled by hereditary factors as is the plumage color and pattern. On the other hand, it is a character which is rather particularly sensitive to environmental influences in *one* direction, namely, downward. I can breed a flock of birds which I *know* will be high winter layers if properly fed, housed and managed. But if these birds are starved, housed in a damp cold place and otherwise maltreated they will lay but a few if any eggs. Under such conditions the genotypic condition would be swamped by the environment. It was this sort of thing I had in mind when I made the statement that Castle quotes. It should be particularly noted, however, that this is a somewhat one-sided matter. I can (because I have done so) breed a flock of pullets lacking totally the factors for winter production. With such birds nothing can be done in the way of feeding or management which will make them lay before some time in February or March when the spring cycle begins.

Now all my work on fecundity has been done in a public institution. Egg production is a commercially valuable thing. We have had to submit the results of our breeding operations, in the shape of the birds themselves, to the practical test of farmers, poultrymen, etc. In doing this there has always been vividly before my mind the fact that unless the birds were given proper feed and care, no matter what the genes they carried, they would not lay many eggs.

On the other hand the *degree* of expression of the character in birds carrying the factors for high fecundity may be favorably influenced by exceptionally favorable circumstances, though the possible effects in this direction are, according to my observations, much smaller in amount than in the opposite direction.

While Castle's comments on the unfortunate sentence under discussion are technically perfectly legitimate, I do not think he is quite fair to the essential underlying point of genetic epistemology, namely, the impossibility of judging the genetic constitution by the somatic appearance.

This of course is the reason for the progeny test. I do not think I am in any sense exaggerating if I say that it is one of the chief results of the Mendelian method of studying inheritance to show that in many cases and for many characters it is impossible, in the absence of a progeny test, to be sure of the genetic constitution of the individual from an examination of the soma alone. I fancy that if I cared to be fussily nasty in my controversial methods I could cite page after page from Professor Castle's Mendelian writings where even he, in order to be quite sure about the genetic constitution of an individual, has had to breed it. This is all I mean by the progeny test. Why am I and my fowls held up to scorn and ridicule because I say that it is frequently impossible to tell the genetic constitution of a fowl with respect to fecundity without breeding it? Surely fecundity in poultry and coat color in rats only differ in this respect in degree, *if they differ at all,* not in kind. I think if any one will read pp. 604 and 605 of my last NATURALIST paper, which is the immediate objective of Professor Castle's attack, he will have to admit that the interpretation which I give of the earlier results is not entirely senseless, and might indeed explain them. In any case, it is in thorough accord, methodologically considered, with the very best current Mendelian usage, including that of Professor Castle himself.

IV

This brings us to the most serious phase of Castle's attack, namely that in which he denies the validity of my conclusions respecting the inheritance of the character fecundity in fowls.

On the top of p. 716 he asserts that I "assume" that two Mendelizing factors are concerned in the inheritance of fecundity, "but without any sufficient published evidence for either conclusion." As I have published[3] many pages of evidence in demonstration of my conclusions on this point, one can only infer from this statement of

[3] In particular in the *Journal of Experimental Zoology,* Vol. 13, 1912.

Castle's that he regards that evidence as totally worthless. It has not so appealed to other workers.[4] Furthermore I think it can be shown that methodologically my treatment of the problem of inheritance of fecundity stands on precisely the same plane as Mendelian work in general, and Professor Castle's Mendelian work in particular. This I shall now try to do.

The essence of a test of a Mendelian hypothesis lies in this: the genetic constitution of the parents of an array of offspring necessitates that the individual offspring bearing different segregating characters, or different segregating categories of the same character, shall occur in definite numerical proportions. If the observed numerical proportions of the offspring agree, within the limits of error due to random sampling, with the proportions expected from the Mendelian hypothesis, then this fact constitutes valid evidence in support of the hypothesis. If no exceptions to this rule appear and a sufficient number of agreeing cases are adduced the hypothesis is regarded as demonstrated. The number of cases necessary to constitute a proof is a purely individual matter. What one person will consider sufficient to establish proof another will not.

Now in the case of fecundity in fowls, Pearl and Surface[5] first established that the Barred Plymouth Rock stock at the Maine Experiment Station was not homozygous in respect of winter egg production, but that it contained, with frequent occurrence, individuals of high fecundity, and also individuals of low fecundity. The *race* not being homozygous with respect to fecundity, it was possible to test the Mendelian inheritance of this

[4] Cf., for example, Morgan, T. H., "Heredity and Sex," New York, 1913, Doncaster, L., "The Determination of Sex," Cambridge, 1914, and Johannsen, W., "Elemente der exakten Erblichkeitslehre," Zweite Ausgabe, Jena, 1913, Plate L. Vererbungslehre, Leipzig, 1913, Brown, E., "Poultry Husbandry," London, 1915, Sturges, T. W., "The Poultry Manual," 3d edition, London 1915.

[5] Pearl and Surface, "Data on the Inheritance of Fecundity Obtained from the Records of Egg Production of the Daughters of '200-Egg' Hens," Me. Agr. Exp. Sta. Bull. 166, 1909.

character *within* the race, without crossing, by the above scheme.

The next step was the definition of the categories of the character winter egg production. From long study of the character I concluded that the natural categories in this strain were (*a*) zero winter production, (*b*) winter production between zero and 30 eggs, and (*c*) a winter production of over 30 eggs. These were chosen as working categories. If any one will turn to p. 719 of Professor Castle's paper and examine Fig. 1, which is there printed, they will find that even he chooses categories of the character with which he is working. Nowhere have these ever been quantitatively defined; nowhere has he ever presented any evidence that the step from his rat grade + 1 (for example) to his grade + 2 represents a more or less inclusive category than a difference in winter production of from 0 to 30 eggs. Professor Castle reads us a beautiful little homily about Mendel's peas. But I am not clear that either Mendel or Castle has shown that the amount of variation *within* the category "yellow" is less than the amount of variation within my fecundity category of "under 30." From the only study which has ever been made of the matter, Weldon's,[6] I should certainly conclude that the category "under 30" in winter egg production carries within itself distinctly *less* variation than the category "yellowness" in peas. Castle's assertion about my fecundity categories ill becomes one whose work in genetics has dealt almost without a single exception with non-quantitatively defined Mendelian categories. Of course, as a matter of fact, he knows, I know, and everybody knows that the variations within the Mendelizing category are of no significance so far as the Mendelian result is concerned. I happen to have observed, for example, that there are at least four genetically distinct rose combs in poultry. Yet they are all *rose;* any of them crossed with single gives a 3 : 1 ratio in F_2.

[6] Weldon, W. F. R., "Mendel's Laws of Alternative Inheritance in Peas," *Biometrika*, Vol. I, pp. 228–265, 1902.

Having chosen these categories of the character fecundity because they appeared to represent natural divisions, I proceeded to show for hundreds of matings the distribution of the progeny when individual females whose performance fell into one or another of the categories were mated to particular males. This was done both for the pure bred Barred Rocks and for crosses. The results at once showed that definite ratios were appearing with regularity and constancy. Further analysis showed that a Mendelian hypothesis which postulated two factors, one sex-linked and the other not, accounted for all the facts.

If all this does not conform to the classic canons of Mendelian experimentation, I am sure I do not know what does.

V

Castle charges me with suppressing data. There are just two things which I wish to say regarding this charge. The first is that I shall publish the complete raw data of my work on the inheritance of fecundity when I have finished my own study of these, and not sooner. I am using this material for the study of various problems. There appears to be no reason why I should make valuable original records public property until such time as I have finished my own analysis of them. If Professor Castle will examine my published papers he will find that in lines of work which I am finishing and leaving, complete raw data are published (cf. for example "A Biometrical Study of Egg Production in the Domestic Fowl," Parts I to III).

In the second place I wish to say that so far as any question of concealment is concerned Professor Castle, or any of his students, will be very welcome to come to the laboratory at any time, for as long as they like, and make any examination of the original record books in connection with published results and conclusions.

There is one further point which needs consideration concerning the charge of suppression of pertinent facts. An important reason, I think, why Professor Castle's own interpretation of his rat selection experiment has not been

freely and universally accepted by workers in genetics lies in the fact that he has never presented his results in such a form that any other interpretation of the data could by any chance be tested. There is, from the methodological standpoint, only one way in which an adequate test can be made as to whether any observed change in the composition of a population is the result of a sorting, or of true germinal change, or an adequate idea gained of *how* the change came about. This is the method of individual pedigree analysis. Only one extensive mass selection experiment has ever been analyzed in this way, and that is in Surface's[7] discussion of the Illinois corn results. The Hagedoorns[8] called Castle's attention two years ago to the necessity of individual pedigrees before any just opinion could be formed as to the meaning of the data. To paraphase Castle's damning indictment of the present writer I may be permitted to call attention to the fact that, so far as concerns the individual pedigree of his rats, "information is denied us" by Castle.

In bringing to a close this part of the discussion I wish to emphasize that, in spite of Castle's assertion to the contrary, any unprejudiced person who will take the trouble to examine the facts will find that, so far as concerns methods of dealing with the data and presenting them for publication, the method of their Mendelian analysis, the method of presenting the results of selection experiments by a series of averages, and other matters of method, my work with fecundity in fowls exactly parallels at every point Castle's work with hooded rats, and is in every way, so far as I am able to judge, exactly as critical as his. His experiments are more extensive in scope than mine, and the character fecundity is a more difficult one to deal with, but so far as methodology is concerned the two researches stand on precisely the same footing. I have not

[7] Surface, F. M., "The Result of Selecting Fluctuating Variations." Data from the Illinois Corn Breeding Experiments. IV[e] Conf. int. de Gen., pp. 221–256, 1911.

[8] Hagedoorn, A. L. and A. C., "Studies on Variation and Selection," *Zeitschr. f. ind. Abst.—und Vererbungslehre*, Bd. XI. pp. 145–83, 1914.

lumped the data any more, nor have I "suppressed" data any more than he has. On the contrary I have published a great deal of exact data, in a series of papers from this laboratory, regarding the character fecundity, its normal variation, etc.

VI

The next point which Castle makes is that the changes which occurred in mean flock production during the sixteen years, for which figures were given in the paper which he criticizes, were probably due to environmental, or at least to non-genetic effects. In making this point he calmly disregards all that I have ever published about the experiments, the means taken to be sure that environmental effects were not mistaken for genetic, etc., and proceeds in his discussion as though all my work on the subject had been absolutely uncritical and that I had never given a thought to checking the correctness of the results. In the first place he notes the changes in the numbers of birds on which the average in different years are based, and points out that these numbers change in a roughly inverse direction to the means. He then says:

> Has not the better environment and lessened competition of small numbers possibly something to do with the result?

They have not. Had Professor Castle been less eager to demolish these fecundity results he might have noted that I have repeatedly stated that since 1908 *all birds in these experiments have been kept in flocks of the same size,* namely 125 birds per flock. The number of such flocks has at times varied, but not the number in each flock[9] except by very small numbers, such as resulted from losses by death, the necessity occasionally of putting a few extra birds in a pen for a brief period and similar very minor

[9] To prevent any mental strain in reconciling the above statement with the third column of Table I, p. 599, in my NATURALIST paper, let me hasten to say that the pens were filled out, if the number of Barred Rocks in the selection experiments did not just equal multiples of 125, with birds from other experiments.

fluctuations. In the first four years (1899–1900, 1900–1901, 1901–1902, 1902–1903) of the *old* experiment the birds were kept in 50-bird flocks. During the five years following (*i. e.*, to 1908–1909) they were kept in 50, 100, and 150 bird flocks. Just precisely how much (or really how little) difference the size of flock made in average egg production has been fully and minutely analyzed biometrically and published by Pearl and Surface[10] some six years ago. It seems reasonable to suggest that before indulging in fast and loose criticism on such a simple point of fact as this it would become Professor Castle to read the literature respecting the work he is attacking. Since this material seems to have been forgotten it may be well to repeat here that the results showed (Pearl and Surface *loc. cit*, p. 115) that in general there was no significant difference in *winter* production between 50, 100, and 150 bird flocks. In later months of the laying year differences appeared but only in the last month of the winter period (February) was there any significant excess of even 50-bird flocks over the others. Furthermore, besides the material which has already been published regarding the possible influence of environmental factors on the results of these experiments, I have carried out a number of special investigations on different phases of this general question which have not yet been published. For example, I have minutely analyzed the data regarding date of hatching to see whether that might not enter as a significant factor in the interpretation of the results. The data on this question are being prepared for publication now, but it may be said in advance that the results show that date of hatching can not possibly have had anything to do with the rise in average flock production which has occurred between 1908 and 1915.

[10] Pearl and Surface, "A Biometrical Study of Egg Production in the Domestic Fowl. Part I. Variation in Annual Production. U. S. Dept. of Agr., Bu. A. I. Bull. 110, pp. 1–80, 1909. Also the effect of flock size upon *winter* production is specifically discussed in detail in Part II, of the same "Study," pp. 113–117, 1911.

VII

Turning now to the general problem of selection there are certain fundamental matters which it seems to me are in danger of being lost sight of in the rapid shiftings of view point which are an essential part of any general controversial campaign, such as Professor Castle's writings of the last few years would indicate that he engaged in. These are:

1. The pure-line concept has certainly been one of the most useful working tools in the practical breeding of plants and animals that has ever appeared. Particularly in plant breeding the pioneer work at Svalöf, which has been repeated and duplicated on a most extensive scale in plant breeding laboratories all over the world, demonstrates in the most complete manner that, whatever may be happening in the germ-plasm of rats, certainly the germ-plasm of our common cereal crops is in such a state or condition that selection within the pure line is without effect. This is a *fact*, real and definite. It lies definitely at the basis of very extensive commercial seed breeding operations in various different countries. To any one familiar with the extent and stability of the practical applications of the pure-line concept in cereal breeding operations, some of our current discussions of the selection problem seem very academic indeed. Even the justly celebrated magnitude of Castle's rat experiments is scarcely of the same order as the combined and accordant experience of expert cereal breeders throughout the world. Before any one makes up his mind finally about the problem of the efficiency of selection within the pure line it will be well to remember that besides Johannsen's famous, if now in certain quarters somewhat distrusted, beans, there are all the Svalöf oats, wheats, etc., to be reckoned with.

2. No one has ever disputed the power of systematic selection to alter populations, which were not pure-lines. Such alteration may extend the *range* of variation very greatly beyond what it was in the original population.

From a methodological standpoint, however, it is necessary to have a very different sort of evidence from that afforded by changing general population means, such as Castle gives for his rats, and I for fecundity, to prove that the process of selection has been the cause of a change in the absolute somatic equivalent of a particular gene or hereditary determinant.

3. It is just in connection with this last point that there seems to me to be a good deal of unclear thinking and arguing at cross-purposes about the selection problem. Let us examine the logic of the matter symbolically.

Let there be a character A, whose somatic variation in the general population is given by a frequency distribution of area $Z \Sigma_{A_1}^{A_n} A$, where Z is the frequency of occurrence of the somatic state or condition A_1, and so on to Z_n and A_n. Now suppose that selection is practised for the somatic condition A_{40}, but that in the original population A_{38} is the most extreme variation in that direction found to exist. Then for A_{40}, $Z_{40} = 0$, and for A_{38}, Z_{38} is very small. Let it be further supposed that the somatic difference between the A_{38} and A_{39} condition may be of *any* determinate magnitude R. It makes no difference to the logic of the case whether R is large or is extremely minute. Now suppose, as a limiting case, that we assume a gamete-soma correlation of 1, *i. e.*, perfect. Then in the gonads of an individual somatically A_{38}, all the germ cells will bear the factor a_{38}. If two such individuals are bred together the progeny will be somatically A_{38}.[11] Suppose that for m generations the matings are of $A_{38} \times A_{38}$. This is continued selection. Then suppose in the $m + 1$th generation, *ex* $A_{38} \times A_{38}$ parents, appears an A_{39} individual.

Concretely this represents a step in advance in the direction of selection. Let us analyze the possible ways in which this may have happened.

[11] This is precisely the condition which prevails in a pure line of oats, except for purely phænotypic variation, superimposed by environmental factors.

(*a*) First we may assume that A_{38_m} and A_{38_m}, the parents of this $A_{39_{m+1}}$, instead of having a_{38} gametes had a_{39} gametes. This would correspond to what is called a mutation. The gamete-soma correlation has been broken by the appearance of a new kind of gamete different from the parental gametes. There has been a sudden definite change in the germ plasm, such that an a_{38} germ plasm has changed to an a_{39} germ plasm.

(*b*) Or, we may assume that $A_{39_{m+1}}$ was produced by the union of two a_{38} gametes, but that these gametes dedevelop a 39 soma instead of a 38 soma. This assumption leads logically straight to genetic indeterminism, a conclusion which, I think, is repugnant to all that is known regarding the physiology of the hereditary process.

Embracing alternative (*a*) then, we may next inquire as to the possible cause of this sudden change of the germ plasm, by an amount of which the somatic equivalent is R, from a_{38} to a_{39}. If we say that this change has been *caused* by a selection, we can only conclude that the fact that A_{38_1}, A_{38_2} . . . A_{38_m} have been placed in particular cages or apartments to breed, for this is the only physical thing that selection means in this case, has been the *cause* of the germinal change. For by hypothesis there has been no mixing of germ-plasms. We have been practising straight selection of the most extreme somatic individuals, all by hypothesis A_{38}, and each homozygous. It seems to me a misuse of terms to say in such a case as that postulated that *selection* has caused the appearance of the *variation* which it selects, unless we are prepared to say that the physical act of the selection of the individuals for mating physiologically effects the germ plasm. Such an assumption we are all agreed would be nonsense. What has happened in the postulated case is precisely this: a new heritable variation in the direction of selection *has appeared* while selection was in progress. If we say any more than this we are going beyond our facts. If the selectionist would state his results in this form, and

not incessantly harp on the string that "selection *caused*" his results, he would be on logically solid ground and would receive a more respectful hearing from those who place a high value upon clear thinking and sound logic in scientific matters.

Now up to this point in the argument there has been no biological point involved, so far as I can see, to which anybody, whether of the pure line or the selection faith can take exception. Certainly I am perfectly willing to admit that germ-plasm changes do sometimes occur, of all magnitudes from the most minute up. Further no one, I take it, will deny that, having appeared, these variations may be seized upon and preserved by selection. I do desire to emphasize, however, that there is no evidence, as yet, that the selection *causes* the variations.

It may be objected that the postulated case is too simple and leaves out of account too many factors. All this, however, will not affect the logic of the case. Generalized, that logic is as follows: A heritable difference between two individuals or races implies a difference in the germ plasm. The difference in the germ plasm must have made its initial appearance at a definite point of time. At that time the germ plasm *changed* from its previous condition. The cause of that change can not be conceived to be the selection for breeding purposes of the parents bearing the unchanged germ plasm. To assert that the new variation is a result of amphimixis due to mating unlike parents would be, in the present state of genetic knowledge, a ridiculous begging of the question, because, in the first place, by hypothesis in any selection experiment individuals genetically as nearly alike as possible are always mated together, and in the second place, as selection continues homozygosity automatically increases.

The whole fact of the matter is that the assertion that selection *per se* causes changes in the germ plasm, is a wholly new addition to the classic Darwinian selection theory, tacked on quite inadvertently, I believe, by some of the modern exponents of that theory. Darwin never

supposed that selection was a cause of favorable variation. Instead he repeatedly pointed out that the fundamental problem behind natural selection was that of the cause of the variations which selection preserved. That problem remains to-day practically in the same condition that it was left by Darwin. We are no nearer, essentially, now than we were then to knowing the cause of *new* variations. The assertion that new variations are caused by selection is the rankest kind of mysticism plus bad logic.

But if selection of the parents can not be supposed the cause of new variations in the individual, then clearly what selection does, and all it can do, is to change the germinal constitution of a race or population by preserving those individuals in which new variations have appeared, and multiplying them. This is exactly what has been done in the hooded rat experiment, it seems to me on Castle's interpretation of the case. In that experiment every favorable variation in the many thousands of rats has been preserved and the individuals bearing it have been multiplied. Others have been thrown away. The range of the character in the direction of selection has been extended far beyond the original range. But would it have been so extended, or could it have been, if the favorable variations had not appeared for selection, or if, having appeared, they had not been heritable? Suppose one started such an experiment with a character which was in a stable condition and not varying. Take, for example, the single comb of fowls, and attempt by selection from a pure single-combed race to produce a stable rose-combed race by selection alone. Prophecy is dangerous business, but I do fancy one would be a very long time on that job! Characters, so far as I can see, will be altered following selection just in proportion as they are varying genotypically. The cause of the *alteration* is to be sought in the cause of the *variations,* not in the selection only.

I have for some time felt that probably the differences in opinion between the selectionists, as represented by

Castle, and the advocates of the pure-line concept, reduces itself finally very largely to a dispute over the use of words, if both are discussing the same objective facts or experiments. It is repugnant to the logical faculties of the pure-linists to be told that selection is a cause of new variations. On the other hand, I suspect that this particular use of words, which is offensive to our camp, would not be deemed absolutely essential to the making of their case by Castle and his followers. Castle's special *bête noir* appears to be that the pure-linists seem to him to deny the possibility of germinal variation, except it be large in amount (a proper De Vriesian mutation). Now I am in no wise authorized to speak for the pure-line advocates, but I can say for myself, and I venture to think others would agree, that this contention forms no part of the real, genuine pure-line body of doctrine. The followers of the pure-line merely have observed *in fact* that it is not so easy to change all things by a process of selective breeding as it has been to change the pattern of Castle's rats, or the egg production of my fowls. Many characters, and many organisms, when got into a homozygous condition exhibit any germinal variation so rarely as to make any change by the selection of such variation impossible within the limits of finite experimentation. Neither Johannsen nor any followers of his, so far as I am aware, have ever attempted to set any limitations on how big or how little a germinal variation could be.

THE EVOLUTION OF THE CELL. II

By the Late PROFESSOR E. A. MINCHIN, F.R.S.

Even more remarkable than the relation of the chromosomes to cell-reproduction is their behavior in relation to sexual phenomena. In the life-cycles of Metazoa the sexual act consists of the fusion of male and female pronuclei, each containing a definite and specific number of chromosomes, the same number usually, though not always, in each pronucleus. It has been established in many cases, and it is perhaps universally true, that in the act of fertilization the male and female chromosomes remain perfectly distinct and separate in the synkaryon or nucleus formed by the union of the two pronuclei, and, moreover, that they continue to maintain and to propagate their distinct individuality in every subsequent cell-generation of the multicellular organism produced as a result of the sexual act. In this way, every cell of the body contains in its nucleus distinct chromatinic elements which are derived from both male and female parents and which maintain unimpaired their distinct and specific individuality through the entire life-cycle. This distinctness is apparent at least in the germ-cell-cycle of the organism, but may be obscured by secondary changes in the nuclei of the specialized tissue-cells.

Only in the very last stage of the life-cycle do the group of male and female chromosomes modify their behavior in a most striking manner. In the final generation of oogonia or spermatogonia, from which arise the oocytes and spermatocytes which in their turn produce the gamete-cells, it is observed that the male and female chromosomes make a last appearance in their full number, and then fuse in pairs, so as to reduce the number of chromosomes to half that previously present.

In *Aggregata* also Dobell and Jameson have shown that the union of the pronuclei in fertilization brings together two sets each of six chromosomes, and that these then fuse with one another in pairs according to type, that is to say *a* with *a*, *b* with *b*, *c* with *c*, and so on. Analogous phenomena have been demonstrated also in the gregarine *Diplospora*. We have here a difference in detail, as compared with the Metazoa, in that the fusion takes place at the fertilization and not as the first step in the maturation of the germ-cells; but in both cases alike the fusion of chromatin-elements individually distinct and exhibiting specific characteristics is to be regarded as the final consummation of the sexual act, though long deferred in the Metazoan life-cycle.

As Vejdovský has pointed out, there can be no more striking evidence of the specific individuality of the chromosomes than their fusion or copulation in relation to the sexual act. Is there any other constant element or constituent of living organisms exhibiting to anything like the same degree the essentially vital characteristics of individuality manifested in specific behavior? If there is, it remains to be discovered.

I come now to the question of the permanence and immortality, in the biological sense of the word, of the chromatinic particles, which may be summarily stated as follows: the chromatinic particles are the only constituents of the cell which maintain persistently and uninterruptedly their existence throughout the whole life-cycle of living organisms universally.

I hope I shall not be misunderstood when I enunciate this apparently sweeping and breathless generalization. I am perfectly aware that in the life-cycle of any given species of organism there may be many cell-constituents besides the chromatin-particles that are propagated continuously through the whole life-cycle; but cell-elements which appear as constant parts of the organization of the cell throughout the life-cycle in one type of organism may be wanting altogether in other types. With the exception

of the chromatin-particles there is no cell-constituent that can be claimed to persist throughout the life-cycles of organisms universally. To take some concrete examples; the cytoplasmic grains known as mitochondria or chrondriosomes have been asserted to be persistent elements throughout the germ-cycle of Metazoa, and the function of being the bearers of hereditary tendencies has been ascribed to them. But Vejdovský[22] flatly denies the alleged continuity in cases investigated by him, and though chrondriosomes have been described in some Protozoa, there is no evidence whatever that they are of universal occurrence in Protista. Centrosomes, intranuclear or extranuclear, have been stated to be constant cell-components in some organisms; whether that is true or not it seems quite certain that in many organisms the cells are entirely without centrosomic bodies of any kind, as for example in the whole group of Phanerogams. So it is with any other cell-constituent that can be named. It may be that this is only the result of our incomplete knowledge at the present time. I am prepared, however, to challenge anyone to name or to discover any cell-constituent, other than the chromatinic particles, which are present throughout the life-cycle, not merely of some particular organism, but of organisms universally.

In this feature of continuity the chromatin-constituents of the cell present a remarkable analogy with the germ-plasm of Metazoa. Just as the germ-cells of Metazoa go on in an uninterrupted, potentially everlasting series of cell-generations, throwing off, as it were, at each sexual crisis a soma which is doomed to but a limited lease of life, during which it furnishes a nutritive environment for further generations of germ-cells; so in the series of cell-generations themselves, whether in the germ-cell-cycles of Metazoa or in the life-cycles of Protista the chromatin-particles maintain an uninterrupted propagative series within a cell-body of which the various parts have a limited duration of existence, making their appearance, flourish-

[22] *L. c.*, pp. 77–89.

ing for a time, and disappearing again. This analogy between the chromatin of cells and the germ-plasm of multicellular organisms becomes still more marked when we find that in many Protozoa the chromatin may undergo a specialization into generative and trophic chromatin, the former destined to persist from one life-cycle to another, the latter destined to control cell-activities merely during one cycle, without persisting into the next. The differentiation of generative and trophic chromatin is now well known to occur in many Protozoa, and in its most extreme form, as seen in the Infusoria, it is expressed in occurrence of two distinct nuclei in the cell-body.

To recapitulate my argument in the briefest form; the chromatinic constituents of the cell contrast with all the other constituents in at least three points: physiological predominance, especially in constructive metabolism; specific individualization; and permanence in the sense of potential biological immortality. Any of these three points, taken by itself, is sufficient to confer a peculiar distinction to say the least, on the chromatin-bodies; but taken in combination they appear to me to furnish overwhelming evidence for regarding the chromatin-elements as the primary and essential constituents of living organisms, and as representing that part of a living body of any kind which can be followed by the imagination, in the reverse direction of the propagative series, back to the very starting-point of the evolution of living beings.

In the attempt to form an idea as to what the earliest type of living being was like, in the first place, and as to how the earliest steps in its evolution and differentiation came about, in the second place, we have to exercise the constructive faculty of the imagination guided by such few data as we possess. It is not to be expected, therefore, that agreement can be hoped for in such speculations; it would indeed be very undesirable, in the interests of science, that there should be no conflict of opinion in theories which, by their very nature, are beyond any possibility of direct verification at the present time. The

views put forward by any man do but represent the visions conjured up by his imagination, based upon the slender foundation of his personal knowledge, more or less limited, or intuition, more or less fallacious, of an infinite world of natural phenomena. Consequently such views may be expected to diverge as widely as do temperaments. If, therefore, I venture upon such speculations, I do so with a sense of personal responsibility and as one wishing to stimulate discussion rather than to lay down dogma.

To me, therefore, the train of argument that I have set forth with regard to the nature of the chromatinic constituents of living organisms appears to lead to the conclusion that the earliest living beings were minute, possibly ultramicroscopic particles which were of the nature of chromatin. How far the application of the term chromatin to the hypothetical primordial form of life is justified from the point of view of substance, that is to say in a biochemical sense, must be left uncertain. In using the term chromatin I must be understood to do so in a strictly biological sense, meaning thereby that these earliest living things were biological units or individuals which were the ancestors, in a continuous propagative series, of the chromatinic grains and particles known to us at the present day as universally-occurring constituents of living organisms. Such a conception postulates no fixity of chemical nature; on the contrary, it implies that as substance the primitive chromatin was highly inconstant, infinitely variable, and capable of specific differentiation in many divergent directions.

For these hypothetical primitive organisms we may use Mereschkowsky's term biococci. They must have been free-living organisms capable of building up their living bodies by synthesis of simple chemical compounds. We have as yet no evidence of the existence of biococci at the present time as free-living organisms; the nearest approach to any such type of living being seems to be furnished by the organisms known collectively as Chlamydozoa, which up to the present have been found to occur

only as pathogenic parasites. In view, however, of the minuteness and invisibility of these organisms, it is clear that they could attract attention only by the effects they produce in their environment. Consequently the human mind is most likely to become aware in the first instance of those forms which are the cause of disturbance in the human body. If free-living forms of biococci exist, as is very possible and even probable, it is evident that very delicate and accurate methods of investigation would be required to detect their presence.

I am well aware that the nature and even the existence of the so-called Chlamydozoa is uncertain at the present time, and I desire to exercise great caution in basing any arguments upon them. In the descriptions given of them, however, there are some points which, if correctly stated, seem to me of great importance. They are alleged to appear as minute dots, on the borderline of microscopic visibility or beyond it; they are capable of growth, so that a given species may be larger or smaller at different times; their bodies stain with the ordinary chromatin-stains; and they are stated to reproduce themselves by a process of binary fission in which the body becomes dumbbell-shaped, appearing as two dots connected by a slender thread, which is drawn out until it snaps across and then the broken halves of the thread are retracted into the daughter-bodies. This mode of division, strongly reminiscent of that seen in centrioles, appears to me to permit of certain important conclusions with regard to the nature of these bodies; namely, that the minute dot of substance has no firm limiting membrane on the surface and that it is of a viscid or semi-fluid consistence.

If it be permissible to draw conclusions with regard to the nature of the hypothetical biococci from the somewhat dubious, but concrete data funished by the Chlamydozoa, the following tentative statements may be postulated concerning them. They were (or are) minute organisms, each a speck or globule of a substance similar in its reactions to chromatin. Their substance could be described

as homogeneous with greater approach to accuracy than in the case of any other living organism, but it is clear that no living body that is carrying on constructive and destructive metabolism could remain for a moment perfectly homogeneous or constant in chemical composition. Their bodies were not limited by a rigid envelope or capsule. Reproduction was affected by binary fission, the body dividing into two with a dumbbell-shaped figure. Their mode of life was vegetative, that is to say, they reacted upon their environmental medium by means of ferments secreted by their own body-substance. The earliest forms must have possessed the power of building up their protein-molecules from the simplest inorganic compounds; but different types of biococci, characterized each by specific reactions and idiosyncrasies, must have become differentiated very rapidly in the process of evolution and adaptation to divergent conditions of life.

Consideration of the existing types and forms of living organisms shows that from the primitive biococcal type the evolution of living things must have diverged in at least two principal directions. Two new types of organisms arose, one of which continued to specialize further in the vegetative mode of life, in all its innumerable variations, characteristic of the biococci, while the other type developed an entirely new habit of life, namely a predatory existence. I will consider these two types separately.

(1) In the vegetative type the first step was that the body became surrounded by a rigid envelope. Thus came into existence the bacterial type of organism, the simplest form of which would be a Micrococcus, a minute globule of chromatin surrounded by a firm envelope. From this familiar type an infinity of forms arises by processes of divergent evolution and adaptation. With increase in size of the body the number of chromatin-grains within the envelope increase in number, and are then seen to be imbedded in a ground-substance which is similar to cytoplasm, apparently, and may contain non-chromatinic en-

closures. With still further increase of size the chromatin-grains also increase in number and may take on various types of arrangement in clumps, spherical masses, rodlets, filaments straight or twisted in various ways, or even irregular strands and networks,[23] and the cytoplasmic matrix, if it is correct to call it so, becomes correspondingly increased in quantity. I will not attempt, however, to follow up the evolution of the bacterial type further, nor to discuss what other types of living organisms may be affiliated with it, as I have no claims to an expert knowledge of these organisms. I prefer to leave to competent bacteriologists and botanists the problem of the relationships and phylogeny of the Cyanophyceæ, Spirochætes, etc., which have been regarded as having affinities with Bacteria.

(2) In the evolution from the biococcus of the predatory type of organism, the data at our disposal appear to me to indicate very clearly the nature of the changes that took place, as well as the final result of these changes, but leave us in the dark with regard to some of the actual details of the process. The chief event was the formation, round the biococci of an enveloping matrix of protoplasm for which the term periplasm (Lankester) is most suitable. The periplasm was an extension of the living substance which was distinct in its constitution and properties from the original chromatinic substance of the biococcus. The newly-formed matrix was probably from the first a semi-fluid substance of alveolar structure and possessed two important capabilities as the result of its physical structure; it could perform streaming movements of various kinds, more especially amœboid movement; and it was able to form vacuoles internally. The final result

[23] See especially Dobell, ''Contributions to the Cytology of the Bacteria,'' *Quart. Journ. Micr. Science*, LVI (1911), pp. 461, 462. I can not follow Dobell in applying the term ''nuclei'' to these various arrangements of the chromatin-grains in Bacteria. Vejdovsky compares them with chromosomes; but there is no evidence that they play the part in the division and distribution of the chromatin-grains which is the special function of chromosomes, as will be discussed in more detail presently.

of these changes was a new type of organism which, compared with the original biococci, was of considerable size, and consisted of a droplet of alveolar, amœboid periplasm in which were imbedded a number of biococci. Whether this periplasm made its first appearance around single individual biococci, or whether it was from the first associated with the formation of zooglœa-like colonies of biococci, must be left an open question.

Thus arose in the beginning the brand of Cain, the prototype of the animal, that is to say, a class of organism, which was no longer able to build up its substance from inorganic materials in the former peaceful manner, but which nourished itself by capturing, devouring, and digesting other living organisms. The streaming movements of the periplasm enabled it to flow round and engulf other creatures; the vacuole-formation in the periplasm enabled it to digest and absorb the substance of its prey by the help of ferments secreted by the biococci. By means of these ferments the ingested organisms were killed and utilized as food, their substance being first broken down into simpler chemical constituents and then built up again into the protein-substances composing the body of the captor.

A stage of evolution is now reached which I propose to call the pseudo-moneral or cytodal stage, since the place of these organisms in the general evolution of life corresponds very nearly to Haeckel's conception of the Monera as a stage in the evolution of organisms, though not at all to his notions with regard to their composition and structure. The bodies of these organisms did not consist of a homogeneous albuminous "plasson," but of a periplasm corresponding to the cytoplasm of the cell, containing a number of biococci or chromatin-grains. Thus their composition corresponded more clearly to that of plasson as conceived by Van Beneden, when he wrote: 'Si un noyau vient à disparaître dans une cellule, si la cellule redevient un cytode, les éléments chimiques du noyau et du nucléole s'étant repandus dans le protoplasme, le plasson se trouve

de nouveau constitué.' If we delete from this sentence the word "chimiques" and also the words "et du nucléole," and substitute for the notion of the chemical solution of the chromatin-substance that of scattered chromatin-grains in the periplasm, we have the picture of the cytodal stage of evolution such as I have imagined it. It should be borne in mind that the ultimate granules of chromatin are probably in many cases ultra-microscopic; consequently they might appear to be dissolved in this cytoplasm when really existing as discrete particles.

In the life-cycles of Protozoa, especially of Rhizopods, it is not at all infrequent to find developmental phases which reproduce exactly the picture of the pseudo-moneral stage of evolution, phases in which the nucleus or nuclei have disappeared, having broken up into a number of chromatin-grains or chromidia scattered through the cytoplasm. We do not know as yet of any Protozoa, however, which remain permanently in the cytodal stage, that is to say, in which the chromatin-grains remain permanently in the scattered chromidial condition, without ever being concentrated and organized into true nuclei; but it is quite possible that some of the primitive organisms known as Proteomyxa will be found to exhibit this condition and to represent persistent Pseudo-monera or cytodes.

The next stage in evolution was the organization of the chromatin-grains (biococci) into a definite cell-nucleus. This is a process which can be observed actually taking place in many Protozoa in which "secondary" nuclei arise from chromidia. It seems not unreasonable to suppose that a detailed study of the manner in which secondary nuclei are formed in Protozoa will furnish us with a picture, or rather series of pictures, of the method in which the cell-nucleus arose in phylogeny. To judge from the data supplied by actual observation, the evolution of the nucleus, though uniform in principle, was sufficiently diversified in the details of the process. As one extreme we have the formation of a dense clump of small, separate

chromatin-grains, producing a granular nucleus of the type seen in Dinoflagellates, in Hæmogregarines, and in Diatoms. Amongst the chromatin-grains there may be present also one or more grains or masses of plastin forming true nucleoli. At the opposite extreme a clump of chromatin-grains becomes firmly welded together into a single mass in which the individual grains can no longer be distinguished, forming a so-called karyosome, consisting of a basis of plastin cementing or imbedding the chromatin-grains into a mass of homogeneous appearance. Whatever the type of nucleus formed, the concentration of the chromidia into nuclei does not necessarily involve all the chromidia, many of which may remain free in the cytoplasm.

In the chromidial condition the chromatin-grains scattered in the cytoplasm are lodged at the nodes of the alveolar framework.[24] Consequently a supporting framework of cytoplasmic origin, the foundation of the linin-framework, was probably a primary constituent of the cell-nucleus from the first. In many nuclei of the karyosomatic type it is very difficult to make out anything of the nature of a framework, which, however, in other cases is seen clearly as delicate strands radiating from the karyosome to the wall of the vacuole in which the karyosome is suspended. Probably such a framework is present in all cases, and each supporting strand is to be interpreted as the optical section of the partition between two protoplasmic alveoli.

With the formation of the nucleus the cytode or pseudomoneral stage has become a true cell of the simplest type, for which I propose the term *protocyte*. It is now the starting-point of an infinite series of further complications and elaborations in many directions. It is clearly

[24] Cf. Dobell, "Observations on the Life-History of Cienkowski's *Arachnula*," *Arch. Protistemkunde*, XXXI (1913), p. 322. The author finds that in *Arachnula* each nucleus arises from a single chromatin-grain, which grows to form a vesicular nucleus. Since the fully-formed nucleus contains numerous grains of chromatin, the original chromidiosome must multiply in this process.

impossible that I should do more than attempt to indicate in the most summary manner the various modifications of the cell-type of organism, since to deal with them conscientiously would require a treatise rather than an address, and, moreover, many such treatises exist already. The most conspicuous modifications of cell-structure are those affecting the periplasm, or, as we may now term it, the cytoplasm. In the first place, the cell as a whole takes various forms; primitively a little naked mass of protoplasm tending to assume a spherical form under the action of surface-tension when at rest, the cell-body may acquire the most diverse specific forms maintained either by the production of envelopes or various kinds of exoskeletal formations on the exterior of the protoplasmic body, or of supporting endoskeletal structures formed in the interior. The simple amœboid streaming movements become highly modified in various ways or replaced by special locomotor mechanisms or organs, flagella, cilia, etc., of various kinds. The internal alveolar cytoplasm develops fibrillæ and other structures of the most varied nature and function, contractile, skeletal, nervous, and so forth. The vacuole-system may be amplified and differentiated in various ways and the cytoplasm acquires manifold powers of internal or external secretion. And finally the cytoplasm contains enclosures of the most varied kind, some of them metaplastic products of the anabolic or catabolic activity essential to the maintenance of life, others of the nature of special cell-organs performing definite functions, such as centrosomes, plastids, chromatophores, etc., of various kinds.

With all the diverse modifications of the cytoplasmic cell-body the nucleus remains comparatively uniform. It may indeed vary infinitely in details of structure, but in principle it remains a concentration or aggregation of numerous grains of chromatin supported on some sort of framework over which the grains are scattered or clumped in various ways, supplemented usually by plastin or nucleolar substance either as a cementing ground-sub-

stance or as discrete grains, and the whole marked off sharply from the surrounding cytoplasm, with or without a definite limiting membrane. There is, however, one point in which the nucleus exhibits a progressive evolution of the most important kind. I refer to the gradual elaboration and perfection of the reproductive mechanism, the process whereby, when the cell reproduces itself by fission, the chromatin-elements are distributed between the two daughter-cells.

The chromatin-constituents of the cell are regarded, on the view maintained here, as a number of minute granules, each representing a primitive independent living individual or biococcus. To each such granule must be attributed the fundamental properties of living organisms in general; in the first place metabolism, expressed in continual molecular change, in assimilation and in growth, with consequent reproduction; in the second place specific individuality. As the result of the first of these properties the chromatin-granules, often perhaps ultra-microscopic, may be larger or smaller at different times, and they multiply by dividing each into two daughter-granules. As a result of the second property, chromatin-granules in one and the same cell may exhibit qualitative differences and may diverge widely from one another in their reactions and effects on the vital activities of the cell. The chromatin-granules may be either in the form of scattered chromidia or lodged in a definite nucleus. When in the former condition, I have proposed the term chromidiosome[25] for the ultimate chromatinic individual unit; on the other hand, the term chromiole is commonly in use for the minute chromatin-grains of the nucleus. The terms chromidiosome and chromiole distinguish merely between the situation in the cell, extranuclear or intranuclear, of the individual chromatin-grain or biococcus.

[25] "*Introduction of the Study of the Protozoa,*" Arnold, 1912, p. 65.

SHORTER ARTICLES AND DISCUSSION

INHERITANCE OF CONGENITAL CATARACT

Cataract is the opacity of the eye caused by a faulty formation of the lens. Certain forms of cataract are congenital and hereditary. Other forms which appear later in life may either be hereditary or due to pathological causes.

In the normal eye the delicate fibers which go to make up the lens are glued together along their sides and at their ends where they unite in lines radiating from the poles of the lens to form a completely transparent body. Anything which prevents the perfect conjunction of these fibers causes a defect in the transparency of the lens. This imperfection has been compared by Harman (2) to the white spots in the finger nail, caused by slight injuries to the nail bed, and he has shown it to be correlated with faulty formation of the dental enamel.

There are various causes for the inhibition of proper lens development, and these give rise to different forms of cataract. Only those forms have been considered here which are congenital.

The most common form of congenital cataract is the lamellar, perinuclear or zonular cataract. This manifests itself as a dark circular disk with the density increasing from the center to the perimeter, forming characteristic zones. These zones are flecked by small wedge-shaped dashes arranged regularly in a spoke-fashion about the disk. The disk is located between the nucleus of the lens and the cortex; and is caused by a thickening of the layers at that place.

Discoid cataract is a slight form of the lamellar, less than 4 mm. in diameter, and located at the posterior pole of the lens. The opacity is uniform throughout, but is not easily visible. (It is sometimes confused with anterior polar cataract, of which the origin is not definitely known, but which is not congenital.)

Coralliform or axial cataract, *cataracta fusiformis*, is an opaque line running through the lens from anterior to posterior pole with a spindle-shaped swelling towards the center of the lens.

Anterior and posterior cortical cataract, *cataracta corticallis*, where the opacity takes a more or less geometrical outline,

cataracta punctata, formed by minute white dots scattered uniformly through the lens or grouped in the anterior cortical layers, and other forms of circumscribed, stationary, lenticular opacities which though rare are known to be congenital (3), have also been used in the compilations given here.

Senile cataract, which also seems to be hereditary, and those forms of cataract arising from lesions, diseases of the eyeball, and certain general diseases such as cholera and tetany, have been omitted.

Although congenital and other forms of cataract have long been considered by the medical profession to be influenced by heredity, no definite analysis was made until 1905, when the first paper by Nettleship (1) appeared. Nettleship's data have been the basis of Bateson's (9) conclusion that the abnormality is inherited as a dominant character. Bateson acknowledges that normal parents have produced abnormal children, but these cases he explains as either *origin de novo,* or due to faulty classification of the parents, who in reality may have been slightly affected with cataract.

Davenport (3) has followed Bateson's conclusion in regard to the inheritance of cataract, and makes the eugenic recommendation that unaffected parents from affected stock may marry without fear of producing abnormal children.

In the "Treasury of Human Inheritance" Harman (2) gives one hundred genealogical tables dealing with congenital cataract. Each table represents two or more generations with a detailed account of the condition of each individual in regard to congenital defects of the eye. The data used in this paper have been taken from these tables. Only those families are used in which there is no doubt as to the condition of the parents or the children in respect to the abnormality, and where there is no question as to the total number of children in each family. After discarding all the doubtful cases, and picking a sibship with its parents from the table as a family, there is left a total of one hundred and twenty-five families which are classified into three different categories, as follows: (*A*) both parents normal with at least one abnormal child; (*B*) one parent normal, the other affected with some form of congenital cataract, with at least one abnormal child; (*C*) both parents abnormal, giving only abnormal children.

There are 31 families with both parents normal which give some abnormal children. In a total of 153 children from these

families, 61 are affected with cataract. This suggests that the character is more likely to be inherited as a recessive than as a dominant. Surely it is not possible to explain so many cases as *origin de novo* or as due to faulty classification of the parents.

In the second category given above (B), where one of the parents is affected and the other normal, the number of defective children would be expected to be approximately the same whether the character was inherited as a dominant or a recessive.

If the abnormality is considered as a recessive character, the ratio of 61 affected to 92 unaffected, already spoken of as having been obtained in the first category of families, shows an excess of recessives over the simple Mendelian expectancy for a monohybrid. This is to be expected since the criterion for including any family in the tabulation is the production of at least one abnormal child. In families with a small number of children it is probable that in some cases only normal children are produced in matings of heterozygote by heterozygote which should give, on the average, one fourth recessive. The observed results must then be compared to a modified Mendelian ratio which will allow for the omission of all-normal progenies. Such ratios have been calculated by Apert (4) and by Wright (5). The expected proportions given here are calculated according to the method given by the latter.

The proportion of recessives varies according to the number of children in the family and ranges, for a three-to-one ratio, from 100 per cent. in families with one child to very nearly 25 per cent. in families with fifteen children. The proportion is calculated from the formula

$$X = \frac{1}{4[1 - (\frac{3}{4})^N]},$$

where N is the number of children. Since the criterion for including any family is the production of one abnormal child, all families with one child must have 100 per cent. abnormal children. The proportion decreases, according to the law of chance, as the number of children in the family increases, finally reaching 25 per cent. as the number of children becomes large.

Table I compares the results obtained with the theoretical expectancy, worked out according to this method.

The method used for testing the agreement of the observed result with the theoretical is the one given by Pearson (6) and Elderton (7). It was originally used to test various series of bio-

TABLE I

NORMAL × NORMAL (BOTH HETEROZYGOUS — NN × NN)

Size of Family N	Number of Families	Total No. of Children	Calculated Proportion Recessive X	Calculated Number of Recessives C	Observed Number of Recessives O	$O-C$	$\frac{(O-C)^2}{C}$
1	2	2	1.0000	2.00	2	.00	.000
2	4	8	.5714	4.57	6	1.43	.447
3	6	18	.4324	7.78	13	5.22	3.502
4	5	20	.3657	7.31	8	.69	.065
5	0	0	.3278	.00	0	.00	.000
6	7	42	.3041	12.77	19	6.23	3.039
7	1	7	.2885	2.02	2	− .02	.000+
8	2	16	.2778	4.44	5	.56	.070
9	1	9	.2703	2.43	1	−1.43	.841
10	2	20	.2649	5.30	3	−2.30	.998
11	1	11	.2610	2.87	2	− .87	.263
	31	153		51.49	61		9.225

$N = 10.$
$P = .418$

	Normal	Abnormal	Per Cent. Abnormal
Observed	92	61	40
Calculated	102	51	33
Difference	10	10	7

logical measurements. Attention has been called to the application of this method of testing theoretical Mendelian ratios by Harris (8).

By calculation from the data given in Table I the measure of agreement, or "*P*," is .418. "*P*" is a value ranging from 0 to 1, proportional to the closeness with which the observed facts agree with the theoretical. In this case in four times out of ten, random samplings of similar data would give results deviating more widely from the theoretical. A possible explanation for this rather wide discrepancy will be given later.

The children from matings of normal by abnormal are given in Table II.

A total of 448 children from 90 families is used. 232 children out of the 448 are found to be defective, whereas 238 are expected according to the modified Mendelian ratio.

As before, the criterion for including any family in the tabulation is the production of at least one affected child. Only in this way can the matings of heterozygous dominants, *Nn* (normal), with homozygous recessives, *nn* (abnormal), be distin-

TABLE II

NORMAL × ABNORMAL (NN × NN).

Size of Family N	Number of Families	Total No. of Children	Calculated Proportion Recessive X	Calculated Number of Recessives C	Observed Number of Recessives O	$O-C$	$\frac{(O-C)^2}{C}$
1	9	9	1.0000	9.00	9	.00	.000
2	11	22	.6667	14.67	20	5.33	1.936
3	10	30	.5714	17.14	19	1.86	.201
4	12	48	.5333	25.60	24	−1.60	.100
5	10	50	.5161	25.81	27	1.19	.054
6	8	48	.5080	24.38	23	−1.38	.078
7	11	77	.5039	38.80	40	1.20	.037
8	9	72	.5019	36.14	31	−5.14	.731
9	3	27	.5009	13.52	15	1.48	.162
10	3	30	.5005	15.02	9	−6.02	2.412
11	2	22	.5002	11.00	10	−1.00	.090
12	0	0	.5001	.00	0	.00	.000
13	1	13	.5001	6.50	5	−1.50	.346
	90	448		237.58	232		6.147

$N = 12.$
$P = .862$

	Normal	Abnormal	Per Cent. Abnormal
Observed	216	232	52
Calculated	210	238	53
Difference	6	6	1

guished from the matings of homozygous dominants, *NN*, with recessives. In the former matings approximately 50 per cent. of the children are expected to be abnormal. In the second case only normal children are expected, who should all be heterozygous for the abnormality. It is entirely possible in small families that matings which should give part abnormal offspring might give only normal children; but all these matings are excluded, for no distinction can be made between them and the more usual matings of abnormal with homozygous normal, also giving only normal children.

Having thus excluded part of the data, the modified ratio is calculated as before, except that in this case it applies to a one-to-one ratio, instead of a one-to-three. It ranges from 100 per cent. in families with one child, to very nearly 50 per cent. in families with fifteen children, and is calculated from the formula

$$X = \frac{1}{2[1 - (\frac{1}{2})^N]}.$$

The agreement of observation with expectancy is very close.

"*P*" having a value .862 means that, in nearly nine cases out of ten, other random samplings would deviate more widely from the theoretical.

The critical test as to whether or not congenital cataract can be considered as a simple recessive character lies in the matings of abnormal by abnormal. Families of this kind should have only abnormal children. Only three such matings are available. In two of these, five and two children, respectively, are the total numbers produced; and these are all abnormal. The other case is a doubtful one. Both parents are classified as having discoid cataract; one is given as seriously affected, the other only slightly so. Seven children are given for this mating: two are abnormal, and the others apparently normal. Three of these five died in infancy, so that their classification is doubtful, but there is no question as to the others. Assuming, as Bateson does in his cases, that there has been a faulty classification of the parents, that the parent given as slightly affected is not congenitally defective, but adventitiously so early in life; then this one discrepancy might be conveniently overlooked.

Another explanation may be found, however, in the fact that heterozygous individuals sometimes show the recessive character. Cases are known where a small percentage of heterozygous individuals show the recessive character, although the homozygous and heterozygous dominants are generally indistinguishable. If such is the case here, the slightly affected parent is heterozygous, and the occurrence of normal children is expected.

This assumption also helps to explain the deviation of observed results above the theoretical in Table I, and their deviation below the theoretical in Table II. If heterozygous individuals are sometimes classified as recessives, it would affect the classification of both the parents and the children. A few matings of abnormal (heterozygote showing the recessive character) by normal would be included in the category *B* which rightfully belong in category *A*. Thus matings giving a one-to-three ratio would be included among matings giving a one-to-one ratio. This would tend to reduce the observed results below the expected. A faulty classification of the children would tend to raise the results, but this would not be as strong a deviating factor as when influencing the parents and therefore a number of children. Thus the balance would tend slightly toward a decrease in the regular expectancy; a result which fits well with that obtained in Table II.

In the matings of normal by normal, there is, of course, no opportunity for this error to influence the classification of the parents, since abnormals, whether heterozygous or not, would not come here. But with the children, the number with the recessive character would be raised above the regular expectancy; a result which coincides with that in Table I.

If with more matings of abnormal by abnormal it is found that, with a few exceptions, only abnormal children are given, the evidence that cataract is a recessive character rather than a dominant will be fairly conclusive. It seems rather strange that congenital cataract manifesting itself, as it does, in such different ways, should be determined by a single unit factor. These things, however, must be explained in the simplest possible manner; an attempt to work it out with two or more factors would introduce great complications, and be practically impossible with the data as they have been gathered heretofore. The fact that a recessive character may not be recognized, for it occurs in mass data in a greater proportion than would be expected at first, should be noticed. Finally the approximation of the results obtained with those expected from the single unit factor form the best reason for its acceptance.

That certain geneticists should have laid down eugenic rules based on the inheritance of this character as a dominant is, at the very least, unfortunate. It is not only because of a mistake in the method of inheritance, but such rules should never be made until the exact hereditary processes are positively known, since such practises are likely, not only to bring discredit upon the science, but to injure people who endeavor to follow them in the regulations of their lives.

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HUXLEY AS A MUTATIONIST

ELSEWHERE I have pointed out that Galton[1] held with equal firmness to continuity and discontinuity in variation, and that the American horticulturist and botanist, Thomas Meehan,[2] held clear mutationist conceptions which he supported by accurate observations of variations in many plants. It seems worth while to add a note on the attitude of Huxley with regard to this question.

Whenever Huxley expressed himself on this matter he usually took occasion to say explicitly that he could see no reason why variations should not be discontinuous as well as continuous, and one of the few points on which he differed from Darwin was in ascribing greater significance to such marked changes. Several statements of his position in this matter are found in his volume of essays entitled *Darwiniana*.

Thus he says (p. 77):

> Mr. Darwin's position might, we think, have been even stronger than it is if he had not embarrassed himself with the aphorism "*natura non facit saltum*," which turns up so often in his pages. We believe, as we have said above, that Nature does make jumps now and then, and a recognition of the fact is of no small importance in disposing of many minor objections to the doctrine of transmutation.

Elsewhere (pp. 34, 404) Huxley refers to the well-known Ancon sheep, which originated from a single ram in the flock of a Massachusetts farmer named Seth Wight. The story of this breed of sheep is told in a letter from Col. David Humphreys to Sir Joseph Banks, then President of the Royal Society.[3] The farmer kept a flock of 15 ewes and one ram on the banks of the Charles River, at Dover, Mass., 16 miles from Boston. In 1791 a ram

[1] "Galton and Discontinuity in Variation," AMER. NAT., *48:* 697–699, 1914.

[2] "An Anticipatory Mutationist," AMER. NAT., *49:* 645–648, 1915.

[3] Humphreys, D., 1813. "On a New Variety in the Breeds of Sheep," *Phil. Trans. Roy. Soc.*, 1813: 88–95.

lamb was born having a short length of back and short bandy legs. Seeing an advantage in such an animal owing to its inability to jump fences, it was bred to the flock, the original ram being killed. The first year thereafter two lambs had the peculiarities of their father, and in following years a number more Ancon lambs were produced. The latter when bred together always, with one questionable exception, produced Ancons.

Hence the character was evidently a recessive, having originated from the normal through a negative variation or mutation, presumably in one germ cell. This being the case, the variation must have been carried in a latent or recessive condition for a certain number of generations until inbreeding brought it out in a homozygous form. The original ram which was killed must have been heterozygous for this character, also one at least of the ewes and probably more; for one such heterozygous ewe was necessary to produce the original Ancon ram, and the two Ancons which appeared next year in the back-cross not improbably came from different mothers. It is therefore impossible to say just how long this condition may have been handed on in a "latent" condition before inbreeding brought it out.

With few exceptions, the Ancons showed alternative inheritance when crossed with normal sheep, and (l. c., p. 90).

> Frequent instances have happened where common ewes have had twins by Ancon rams, when one exhibited the complete marks of features of the ewe; the other of the ram.

Incidentally this shows that such twins came from separate ova.

In a flock the Ancon sheep tended to keep together and separate from the normal members of the flock. The breed seems to have attained some popularity, but their flabby subscapular muscles, infirm construction, loose joints, crooked forelegs and awkward gait, while preventing them from jumping fences made them difficult to drive to market. Butchers also found the carcasses smaller and less saleable, so that they were soon supplanted after the introduction of the Merino. They were already scarce in 1813 and afterwards became extinct.

Huxley remarks regarding this case:

> Varieties then arise we know not why; and it is more than probable that the majority of varieties have arisen in this "spontaneous" manner, though we are, of course, far from denying that they may be traced, in some cases, to distinct external influences. . . . But however they may have arisen, what especially interests us at present is, to remark

that, once in existence, many varieties obey the fundamental law of reproduction that like tends to produce like; and their offspring exemplify it by tending to exhibit the same deviation from the parental stock as themselves.

After further discussing the case, Huxley remarks (Op. cit. p. 39):

Here, then, is a remarkable and well-established instance, not only of a very distinct race being established *per saltum,* but of that race breeding "true" at once, and showing no mixed forms, even when crossed with another breed.

Réaumur's case of a Maltese couple having a hexadactylous son, three of whose four children were again hexadactylous, also comes in for Huxley's comment (p. 35 ff.). The following dicta on the subject of variation, from the same volume, are also worth quoting:

Indeed we have always thought that Mr. Darwin unnecessarily hampered himself by adhering so strictly to his favourite "Nature non facit saltum." We greatly suspect that she does make considerable jumps in the way of variation now and then, and that these saltations give rise to some of the gaps which appear to exist in the series of known forms (p. 97).

I apprehend that the foundation of the theory of natural selection is the fact that living bodies tend incessantly to vary. This variation is neither indefinite, nor fortuitous, nor does it take place in all directions, in the strict sense of these words. . . . A whale does not tend to vary in the direction of producing feathers, nor a bird in the direction of developing whalebone (p. 181).

The importance of natural selection will not be impaired even if further inquiries should prove that variability is definite, and is determined in certain directions rather than in others, by conditions inherent in that which varies. It is quite conceivable that every species tends to produce varieties of a limited number and kind, and that the effect of natural selection is to favour the development of some of these, while it opposes the development of others along their predetermined lines of modification (p. 223).

From these and similar statements it appears evident that were Huxley living to-day he could scarcely escape being classed as a mutationist.

R. RUGGLES GATES

UNIVERSITY OF CALIFORNIA

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HYBRIDISM AND THE RATE OF EVOLUTION IN ANGIOSPERMS

Professor EDWARD C. JEFFREY
Harvard University

In responding to an invitation to contribute to the morning program of the American Society of Naturalists, it has seemed to me that a statement emphasizing some of the morphological features of the greatest of all biological problems, the *modus operandi* of the process of evolution, would be of interest to my fellow biologists. The most distinguished as well as the most profound investigator, which our science has yet produced, Charles Darwin, has unequivocally expressed the opinion in the "Origin of Species," that morphology is the soul of natural history. As I am addressing a body of men who call themselves naturalists, my theme will, I hope, not appear unimportant.

The rate of evolution has not been the same at all periods of our earth's history. There is an agreement among those whose knowledge of the vegetable population of earlier eras makes their opinion worthy of serious regard, that the plant kingdom in former times was in a much less rapid condition of evolutionary change than in the present age. Within the limits necessarily assigned to me it is impossible to state all the probable causes of this notable acceleration in the rate of change in plants. I shall touch only upon two aspects of this problem and of these I shall be able to develop but one.

Extremely important factors in the evolution of plants

have unquestionably been the progressive cooling of our earth's surface, as well as those recently recognized secular periodic twilights of the sun god, known as glacial periods. The latter have worked in an exterminating manner and have wiped out well nigh completely whole types of plants and have left the way clear for the unrestricted development of better adapted forms. For example, at the end of the Paleozoic, in the late Permian, we find world-wide evidence of glaciation, which resulted in the virtual extinction of the great cryptogamic forests, which contributed the raw materials of our most abundant coals. With the passing of the arboreal Cryptogams, the Gymnosperms became the predominant element of the world forests in the Mesozoic. At the end of the Cretaceous there was another age of extinction, which wiped out the mass of Gymnosperms and particularly the Conifers. The naked seeded plants, which prevailed in the medieval period of our earth's history, have in the vegetation of to-day been reduced in the number of species to the merest fraction of seed-producing plants; which in the present age are overwhelmingly angiospermous.

From the present standpoint, however, the progressive but not spasmodic cooling of our earth is of even greater importance. Investigations initiated in my laboratories have made it clear that herbaceous Angiosperms have been derived from woody ones as a response to the increasing coldness of terrestrial climates. Plants of this organization are of such efficiency that they are able to go from seed to seed in a few weeks and thus pass through the inclement winter season in a resting stage. The original researches in this direction were undertaken by Professor Eames. The theme in the past two years has undergone a profitable exploitation by other former students in both botanical and geological publications. The origin of the herbaceous type in the Angiosperms has in itself added a notable impetus to the rate of evolution in the group. Whatever hypothesis one adopts as to the mode of the origin of species, it is quite clear that the

multiplication of generations as well as of individuals, rendered possible by the appearance of the herbaceous type of small size and short reproductive cycle, will contribute to the acceleration of evolutionary processes.

A noteworthy feature, which distinguishes the huge aggregation of Angiosperms now inhabiting the surface of the globe (in the neighborhood of one hundred and forty thousand species) from the saved remnant of the Gymnosperms, is their inherent variability. This high degree of variability has naturally made the Angiosperms a very difficult group from the systematic standpoint and has likewise put them in the foreground in connection with discussions as to the origin of species. Two of the oldest tribes of the coniferous Gymnosperms are the pines and and the araucarians. I have had the good fortune to be able to make a careful comparison of structure extending to all important details, between living representatives of these tribes and their predecessors in the Cretaceous of the eastern United States. It is quite clear from these studies that the genus *Pinus* and the genus *Araucaria* in the remote times of the Age of Chalk, differed only in the smallest particulars from their living descendants. The conclusion inevitably follows that the course of evolution here has been very slow. The actual situation corresponds accurately with the data derived from the past. A white pine, compared with an evening primrose or a rose, is relatively constant and invariable.

The remarkable variability of the Angiosperms, as frequently expressed in terms of the difficulty of systematic identification, brings us naturally to the much debated question of the origin of variability. Darwin, as is well known, simply accepted this phenomenon as a fact and did not, after the first, at any rate, attempt to explain the condition in terms of other phenomena. It is interesting, however, to note that in the beginning he was disposed to accept hybridization as the cause of the variability of species and apparently abandoned this belief only because he could find no evidence for its occurrence on a suffi-

ciently extensive scale. Quite recently the view that heterozygosis is responsible for the mutability of species has again been advanced by Lotsy in an interesting article published in the *Archives Néerlandaises.* This author very definitely takes the position that variability in general is due to hybridization, and that true species (not necessarily those of Linnæus and other systematists) are invariable. With this view I am personally in agreement, with the limitation that the statement goes much too far.

It is one of the commonplaces of breeding that the offspring resulting from hybridization is extremely variable and may be characterized by a greater or less degree of sterility. Taking the particular case of the Angiosperms, it is found that when species of lilies, irises, honeysuckles, etc., are crossed, the result is a highly mutable progeny with a greater or less degree of sexual sterility, the latter condition most easily recognized in the microspores or pollen. The main purpose of the present statement is to make it clear to my fellow naturalists that in nature a high degree of variability often exists in the case of the Angiosperms, expressed either in terms of difficulty of systematic determination in view of intergrading forms, or often in the less obtrusive form of multiplication of species in a given genus. This extreme degree of variability is very largely accompanied by the highly significant phenomenon of pollen sterility.

A family of Angiosperms much in the foreground in recent years is the Onagraceæ or Evening Primrose family. In the case of the genus *Œnothera* remarkable conditions have been discovered by De Vries. The plants of *O. lamarckiana,* when grown in large numbers, show a number of individuals, sometimes as high as one twentieth of the total number, markedly different in character from typical *O. lamarckiana.* This phenomenon was at first thought to be peculiar to this species of *Œnothera* and a great deal of importance was consequently attached to clearing up its somewhat dubious systematic position.

Fortunately we are relieved from the uncertainties necessarily connected with this kind of investigation, by the discovery in more recent years that other and perhaps all species of the genus possess the same features. The activity of systematic botanists in recent years in making new species of *Œnothera* is highly significant in the present connection. The exceptional individuals which grow up in cultures of species of *Œnothera* have been termed by De Vries and his disciples "elementary species." The biological world has been asked to believe that in the appearance of these new forms in cultures of *Œnothera,* we have the phenomenon of mutation or the origin of species at a leap. This view of the matter is, however, open to serious question. The species of *Œnothera,* as well as their so-called mutants, are distinguished by a degree of pollen sterility often extreme. This condition has convinced so accomplished a geneticist as Professor Bateson that the so-called elementary species of *Œnothera* are segregates resulting from previous hybridization. This view of the matter is supported by the fact that the products of hybridization are often relatively fixed forms, as indeed has been noted by Brainerd in his extremely interesting observations on hybrid wild violets.

Obviously the question of possible mutation in the genus *Œnothera* entered into a new and biologically more advantageous phase when other species than *O. lamarckiana* came into the discussion. Clearly a still wider view should even more clarify the situation. Two years ago Miss Ruth Holden, who is at present living in Cambridge, England, made the interesting discovery that the common fireweed, *Epilobium angustifolium,* growing wild near Cambridge and also cultivated in the Cambridge botanic garden, was characterized by a large degree of sterility of pollen. She at once generously communicated her discovery to me and at the same time suggested a reason for the condition of pollen found in the English specimens of *Epilobium angustifolium.* I must here remind you that under the genus *Epilobium* are included two distinct

subgenera, namely *Chamænerion,* distinguished, among other features, by its distinct pollen grains; and *Epilobium* proper having its pollen grains in groups of four. *E. angustifolium* belongs to the section *Chamænerion,* and in the southern part of Canada and the Northern States has no allied species except in mountainous regions (*e. g.*, mountainous Quebec and Colorado). Acting on the suggestion supplied by Miss Holden's discovery, Mr. C. A.

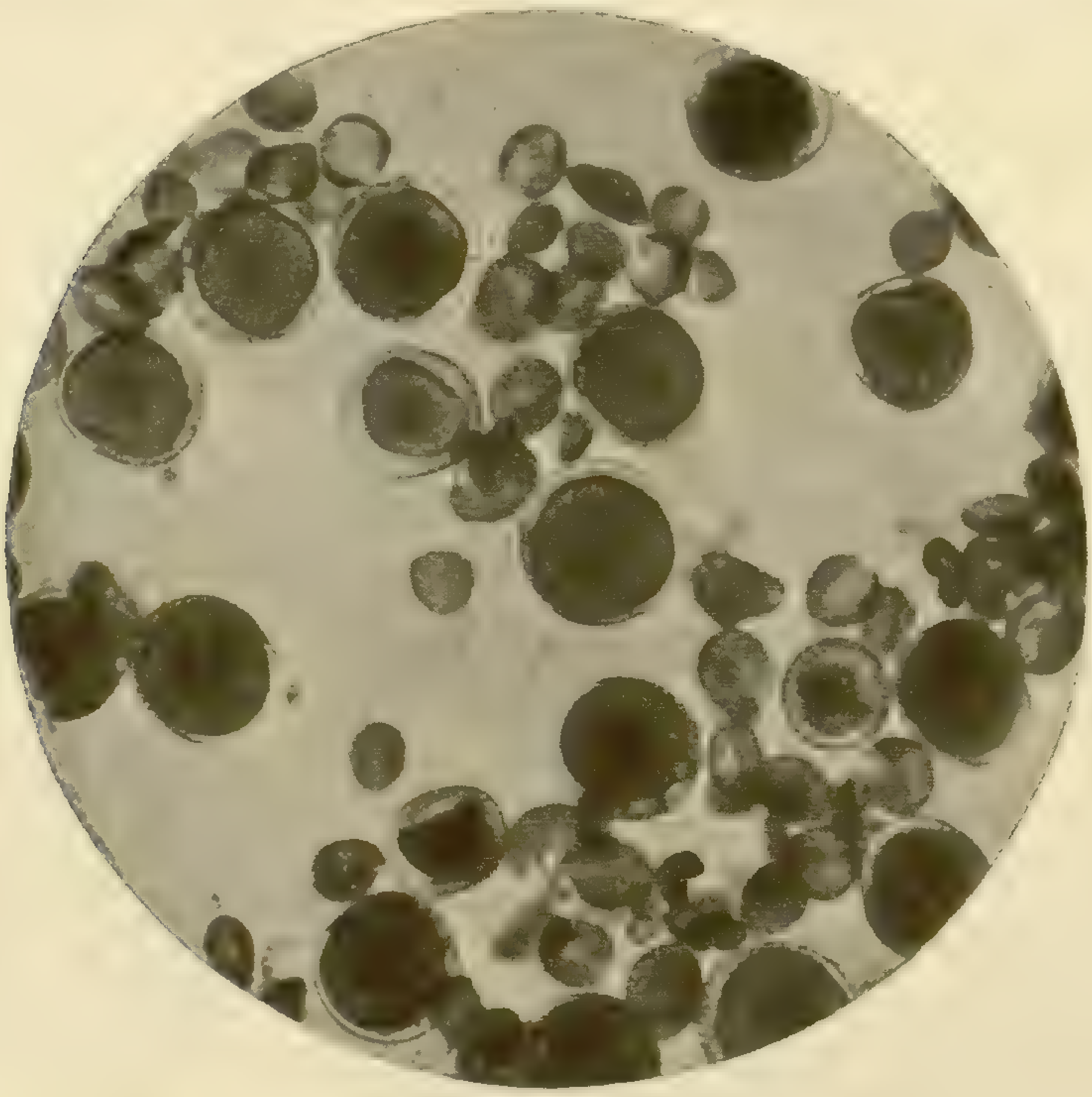

FIG. 1. Pollen of hybrid *Iris germanica.*

Forsaith, one of my graduate students, has investigated the conditions of sterility found in species of *Epilobium* belonging to the section of *Chamænerion.* Through the kindness of the Gray Herbarium of Harvard University he has been able to study some two hundred specimens from various geographic regions. The conditions in *Epilobium* (*Chamænerion*) *angustifolium* in the northern part of its range, where it coincides in distribution with its allied species, *E. latifolium,* are most interesting. Nearly nine tenths of the specimens showed the pollen to be imperfect. In contrast, the material from the southern limits, where *E. angustifolium* does *not* coincide in distri-

bution with *E. latifolium,* are almost uniformly characterized by a high degree of perfection. To be specific, specimens from Ontario, western Quebec, and New Hampshire and Massachusetts show pollen perfectly developed or at most with a few grains disorganized. Mr. Forsaith extended his investigation, again through the courtesy of the Gray Herbarium, to the other genera and species of the Onagraceæ, with similar results. The investigation as a whole will be described elsewhere, but it will be necessary

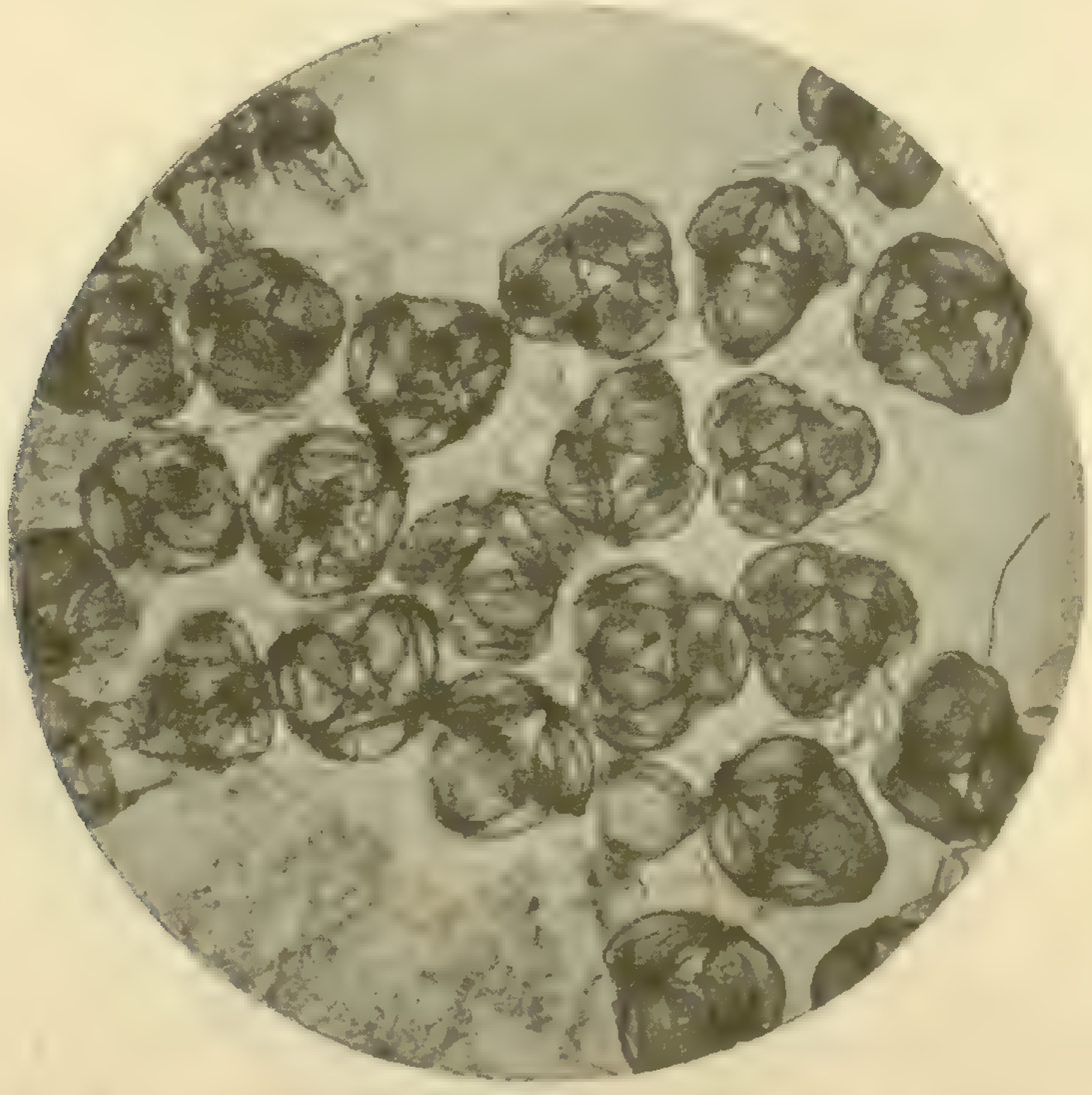

FIG. 2. Pollen of *Zauschneria californica,* a monotypic representative of the Onagraceae.

to consider a few more illustrations in the present connection. There is one quite monotypic species in the order, namely *Zauschneria.* It was found that in this the pollen is practically perfect and the same state of affairs is present in the two geographically limited species of *Gongylocarpus,* one occurring in Vera Cruz and the other on the opposite side of the continent in Lower California. The general situation in the case of the Onagraceæ, a family much in the foreground at the present time by reason of the investigations of De Vries and his disciples, is that monotypic species or those geographically isolated have

perfect pollen and are little characterized by variability; while where the species are numerous and coincident in their range both variability and pollen sterility are conspicuous.

We may now consider another highly variable group, which has not infrequently been called a hybrid family, namely the Rosaceæ. The genera *Rosa, Rubus* and *Cratægus* are notable for the extreme difficulty they have offered from the systematic point of view. Three of my

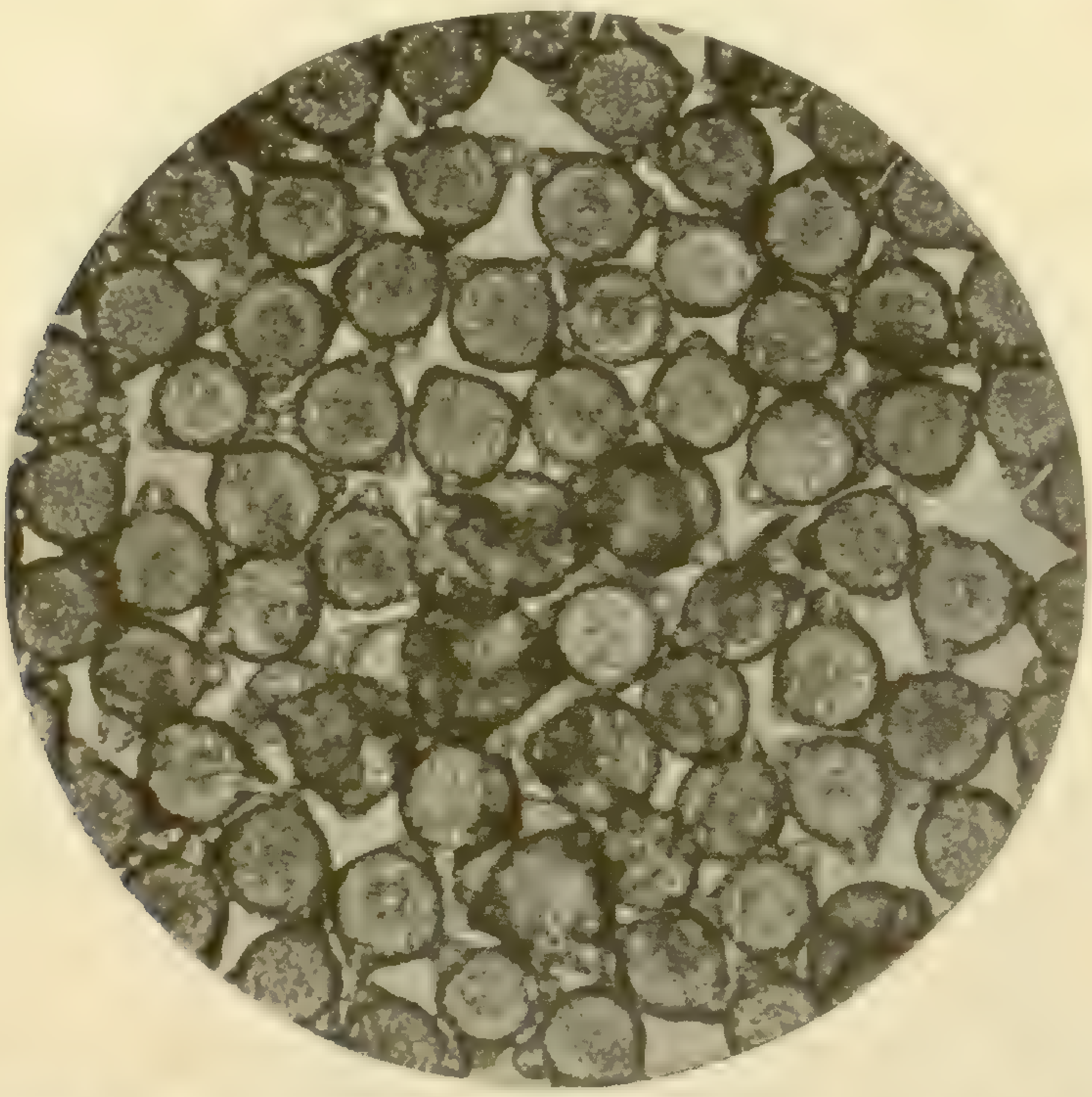

FIG. 3. Pollen of *Chamaenerion* (*Epilobium*) *angustifolium* from Massachusetts.

graduate students have investigated these genera and the results may be conveniently summarized by reference to the genus *Rubus*. In the case of *Rubus*, in regions where it has been exhaustively studied, there is almost no end to the species which may be set up. In Europe, in fact, the species have mounted into the thousands. The situation may for the sake of brevity be considered under three heads. First, there are species which range together and have flowering periods which overlap—a condition common to the mass of our ordinary *Rubi*. In *Rubus villosus*,

the blackbriar, and *R. strigosus,* the wild red raspberry, both very variable species, the pollen is extremely bad. Where these species occur on islands, however, the pollen is generally much more perfect, probably as the result of isolation. I have noticed, for example, that *R. villosus* and *R. strigosus* from Cape Breton Island have considerably better pollen than that found in the case of continental material of the same two species. What is true of these particular species holds more or less well for a

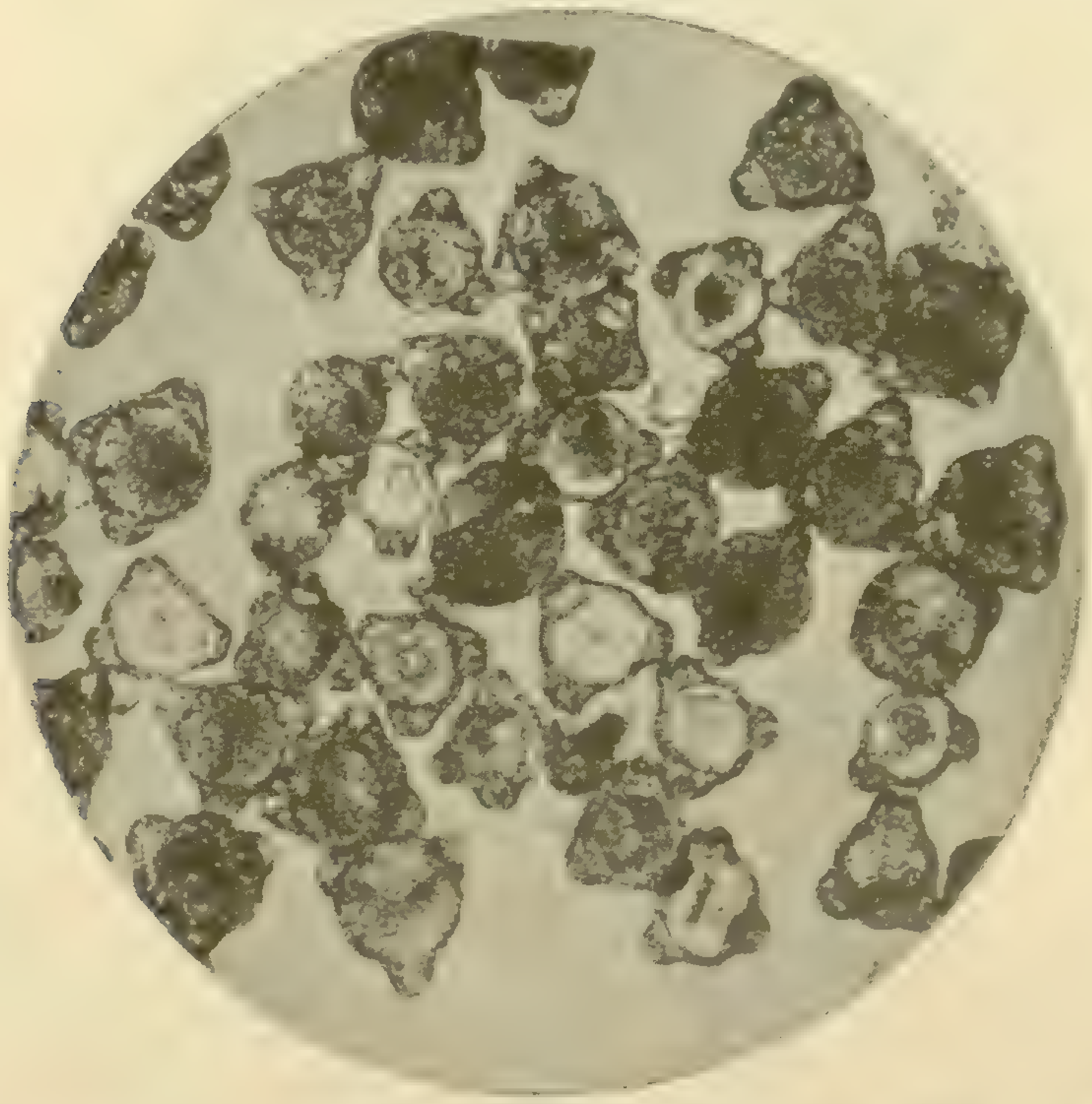

FIG. 4. Pollen from *Chamaenerion* (*Epilobium*) *angustifolium* from the vicinity of Cambridge, England, showing abortive grains.

large number of others of similar range and flowering periods. Next may be considered a species of limited geographic range, namely *R. deliciosus* from the Rocky Mountains. Here the pollen is practically entirely perfect, a few defective elements being occasionally found. Last may be described *R. odoratus,* the so-called flowering raspberry, which blossoms after the mass of other species have shed their flowers. Here, as one might expect, the pollen is highly perfect and practically unmingled with shrivelled grains. A general study of the Rosacæ, which can not even be summarized in the brief

time at my disposal, shows clearly that propinquity, geographical or phenological, is to a large extent correlated with pollen imperfection in the group.

Limitations of time make it necessary to proceed summarily with other illustrations. Next may be cited the Betulaceæ and Fagaceæ. Each of these orders has one strikingly polytypic species, *Betula* in the one case, and *Quercus* in the other. Interestingly enough, it is in these two genera that variability and gametic sterility coincide.

FIG. 5. Pollen of *Rubus deliciosus* from the Rocky Mountains, showing well developed grains.

One might continue at length through the Dicotyledons, but one other example must suffice for this division of the Angiosperms. The Solanaceæ have one huge genus, *Solanum* itself, in which there are nine hundred species. In this genus not only is there extreme variability, but also a large degree of pollen sterility. In the monocotyledonous division we may start with the grasses. Monotypic grasses have perfect pollen, as is illustrated, for example, by the wild rice, *Zizania aquatica*. In the genus *Alopecurus*, with numerous and propinquitous species, on the contrary the pollen conditions frequently indicate gen-

etical contamination. Proceeding to aquatics, in the Potamogetonaceæ, the monotypic *Zannichellia* and *Zostera* have perfectly developed microspores; while *Potamogeton,* with its numerous species, is often distinguished by a large degree of pollen imperfection. Similar statements hold in a like sense in regard to members of the Alismaceæ, Sparganiaceæ, etc.

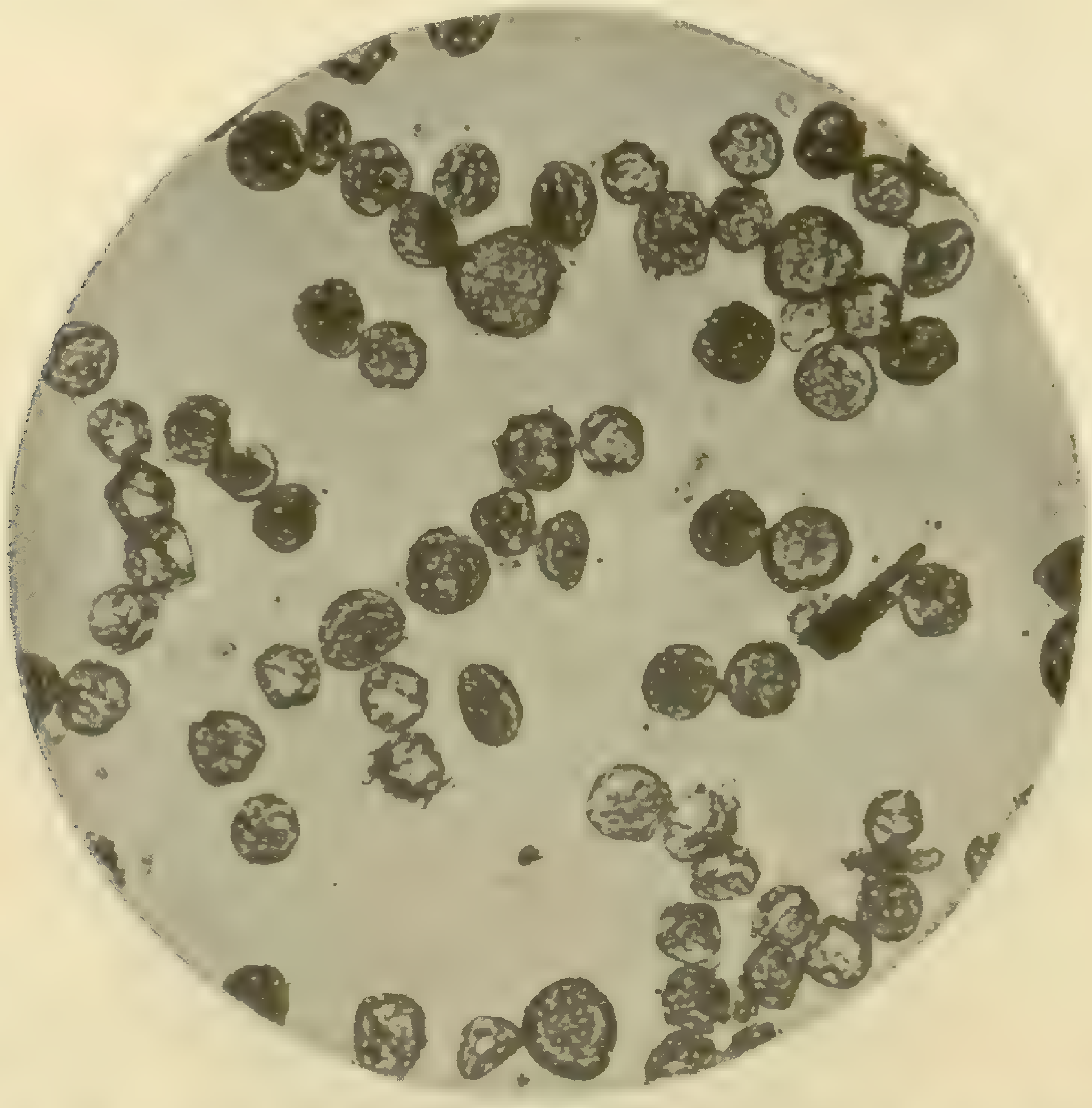

FIG. 6. Pollen of *Rubus villosus* (Blackbriar), showing high degree of imperfection.

The pressure of time compels a summing up of the situation without further references to detailed facts, which will be supplied by publications soon to appear. The general condition in the Angiosperms in contrast to the Gymnosperms is a large degree of variability in the species. Where the species are highly inconstant and cause great difficulty to the systematist, as, for example, in the Onagraceæ, Rosaceæ, Solanaceæ, Birches, Oaks, etc., there is often a large degree of pollen sterility. Where isolation, geographical, phenological or specific, is present the contents of the anther sacs are strikingly perfect in their development. In other words, where interspecific crossing is possible, there is often clear evidence of its presence

in the form of a high degree of variability, accompanying a considerable manifestation of sterility in the gametic cells, particularly the pollen. In the numerous species of *Rosa* or *Œnothera,* we find in regard to both variability and the phenomenon of sterility, a marked contrast to the also numerous species of the very old genus *Pinus.* In *Pinus* there is practically no imperfection in the development of the microspores, even in exotic species, and the species are very clearly marked and constant.

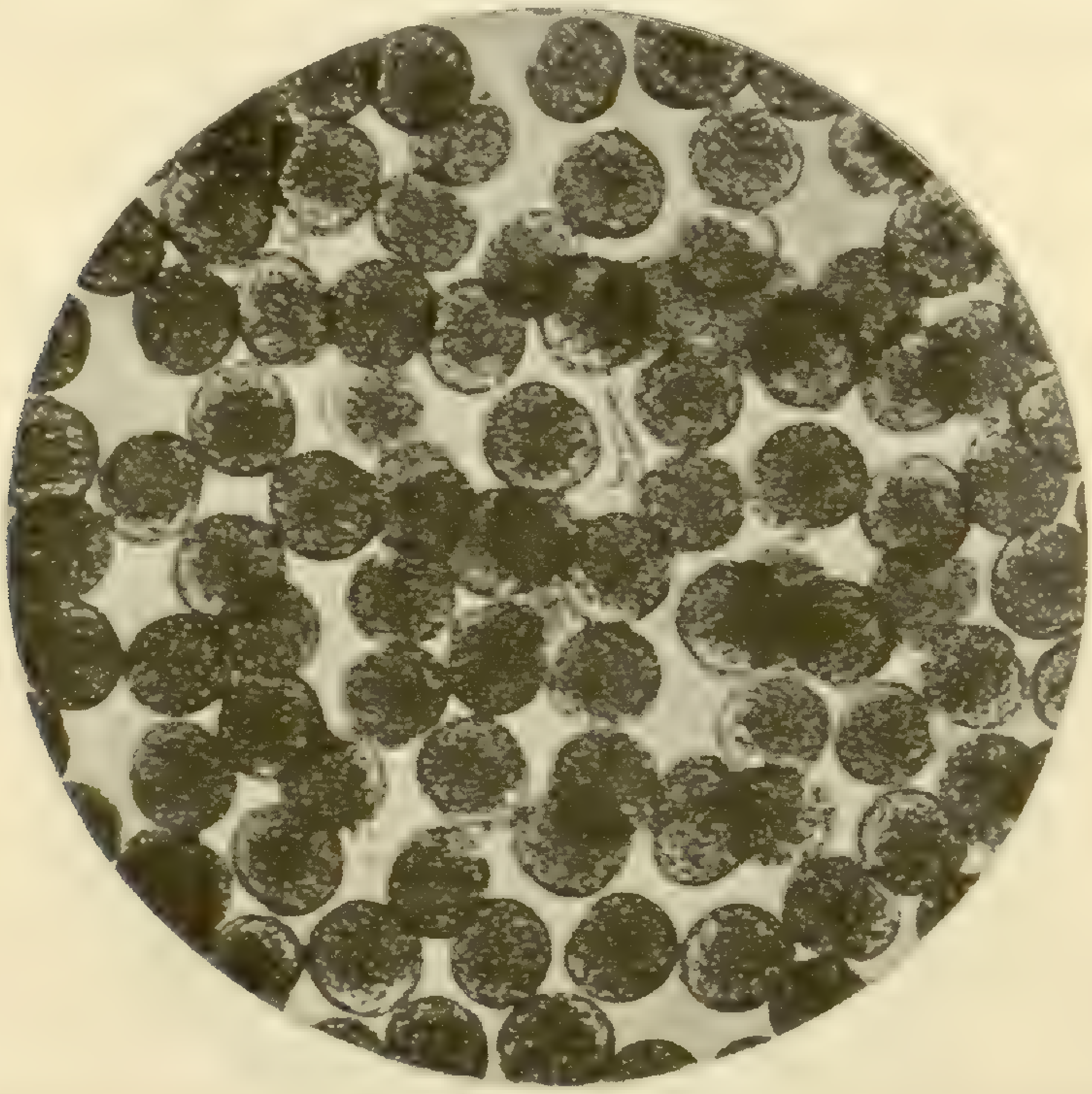

FIG. 7. Pollen of *Zizania aquatica,* a genus with few isolated species.

If associated variability and gametic sterility are reliable indications of hybridization, then it becomes clear that the Angiosperms, unlike the Gymnosperms and the mass of the vascular Cryptogams, are often characterized by heterozygosis. It has been recently suggested that pollen imperfection is not so much an evidence of hybridization as of mutability. This criticism appears to fail for various reasons. First, for nearly a hundred years practically all students of hybridization in plants have noted pollen sterility and imperfect development of the seed as peculiar characteristics of hybrids. Secondly,

in genera with often highly sterile species, such as *Rubus,* the species which are isolated for any reason from the rest have either perfect pollen or manifest a much less marked degree of sterility. An objection urged by De Vries to gametic degeneracy as a criterion of hybridism needs apparently only to be stated to supply its own refutation. The distinguished plant physiologist of Amsterdam, in a recent article in which he criticizes the writer's

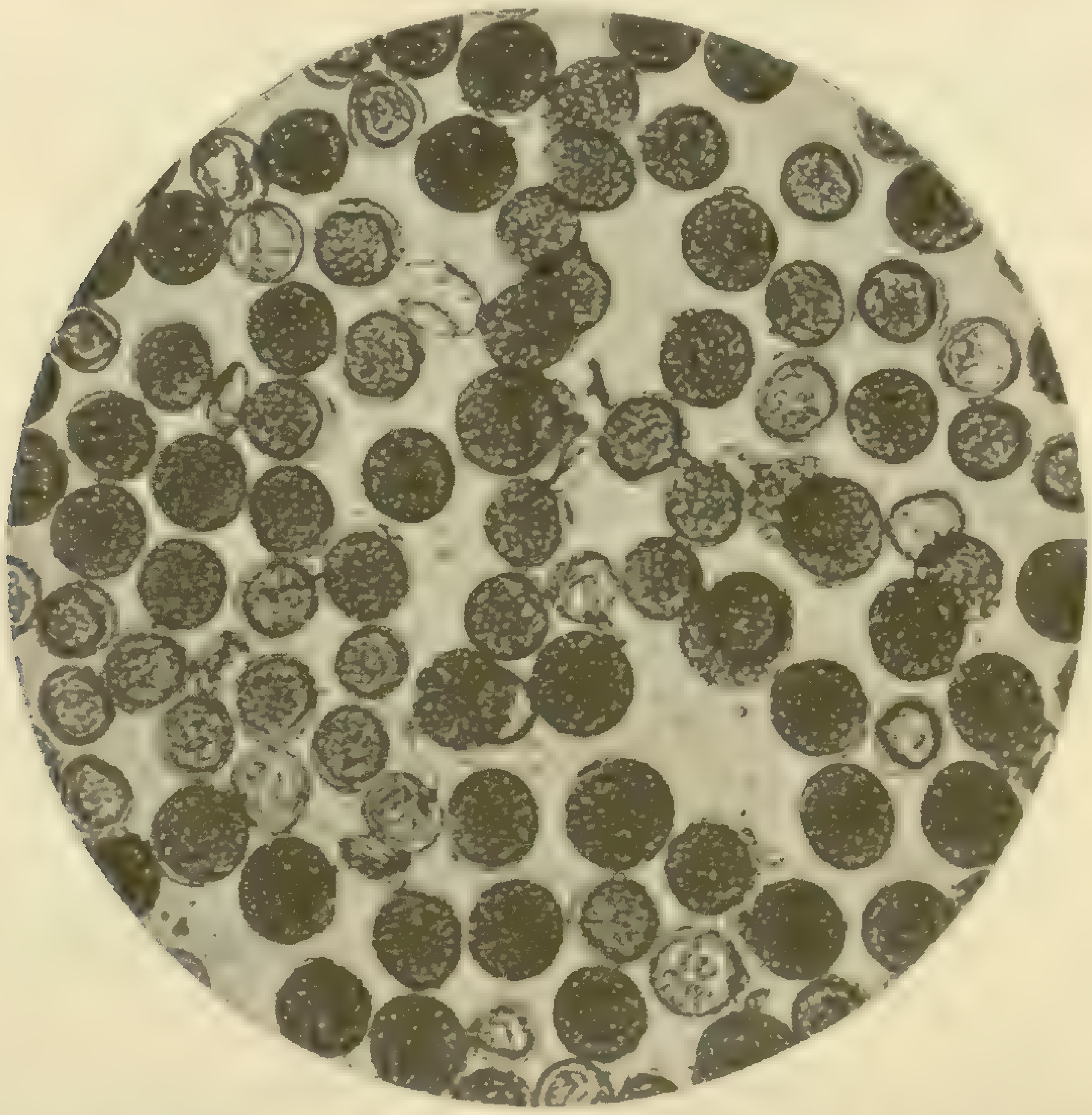

FIG. 8. Pollen of *Alopecurus pratensis*, showing high degree of imperfection which may occur in a polytypic genus.

attitude in regard to the intimate relation between defective pollen, hybridization and so-called mutation, somewhat superciliously, states that the degeneracy of spores in connection with the development of the megaspores of the heterosporous vascular Cryptogams (and one might add the seed plants as well) might with equal validity be regarded as evidence of hybridism in the megasporic sporangia. One has only to carry De Vries's argument to its logical conclusion to prove its entire fallacy. Since microsporic sporangia (in which there is no spore degeneration apart from hybridism) and megasporic sporangia occur ordinarily or at least primitively on the same plant,

it follows that so far as the phenomenon of spore degeneration is concerned some sporangia (the megasporangia or seeds) are of hybrid origin and others (the microsporangia or anthers) are not. The logical absurdity of this conclusion will be clear to every one.

There seems to be no question on the basis of the well-established criteria of hybridism, that many Angiosperms present clear indications that their species are of heterozygous origin. Since one of the most efficient methods of inducing variability in connection with the development of improved varieties of plants is hybridization, often on a very large scale, it seems not unreasonable to regard spontaneous hybridization in the Angiosperms (the evidences for which are so numerous and so impressive) as having an incalculably large effect on their rate of evolution. There is, however, apparently no reason for assuming a similar condition in the Gymnosperms and the vascular Cryptogams. The great and indeed overwhelming advantage which the Angiosperms have secured in the struggle for existence over the lower groups of vascular plants is apparently connected in an intimate way with hybridism on the one hand and the development of herbaceous types (in response to progressive climatic refrigeration) on the other. If this conclusion is correct we must reject the assumption of universal hybridism as the sole cause of variation put forward by Lotsy as much too sweeping. Small variations unquestionably characterize the Gymnosperms, and in the course of long geological time have availed in the absence of competition from heterozygous types, with a much greater range of variability and consequently a higher potentiality of evolution. It is obviously impossible for the homozygous Conifers to make headway against the characteristically heterozygous Angiosperms. The small variations of homozygous stocks clearly prevailed in the earlier history of our earth, while the more rapid changes which have ensued in later times are correlated, so far as plants are concerned, at any rate, with marked physiographic and

climatic differentiation, and most important of all with the phenomenon of heterozygosis.

In conclusion the situation may be summarized. The phenomenon of variation in the older types of plants is still unexplained and must apparently be accepted as an ultimate characteristic of living matter. In the case of those groups of plants, which have achieved predominance under the present climatic conditions of our earth, hybridism has clearly played a large rôle in the acceleration of the processes of evolution. The peculiar conditions presented by the species of *Œnothera,* which have been put forward by De Vries in favor of his mutation hypothesis, are obviously only a particular case of the manifestation of the natural hybridism, which is so widespread a feature of the Angiosperms. The mutation hypothesis has suffered a process of rapid disintegration of late and it is increasingly clear on the botanical side that where the term mutation is used it ordinarily indicates changes which are the result of previous hybridization. Concerning the Animal Kingdom the trend of opinion is apparently setting equally strongly against mutation. My zoological colleague, Professor Castle, has recently declared himself in no uncertain terms against the hypothesis of mutation, an expression of opinion not the less convincing because he originally held the view that mutation was a necessary pendant to Mendelism. He is now able to explain to himself the appearance of new characters as a result of the summation of small variations, which is essentially the Darwinian position.

A FURTHER ANALYSIS OF THE HEREDITARY TRANSMISSION OF DEGENERACY AND DEFORMITIES BY THE DESCENDANTS OF ALCOHOLIZED MAMMALS. II

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The Influence of Internal and External Factors on the Quality of the Offspring

Table II gives the relationship between the size of the litters and the mortality of the descendants from different combinations. It brings out in a way the variable internal and external factors to be considered in interpreting the conditions of the members of the numerous litters of animals. The external factor considered in the table is one of nutrition or environment, depending upon the number of young developed in the uterus at any one time. The table indicates the influence of an internal factor, the germ plasms concerned in mating related or non-related animals. Four combinations are considered: pairs of normal non-relatives, pairs of alcoholic non-relatives, pairs of normal relatives, and pairs of alcoholic relatives.

The first vertical column shows that in mating together normal non-related guinea pigs of the stocks used in these experiments the average litter contains 1.96 individuals. Fifty-one and eleven hundredths per cent. of the young were found in litters of two, and 20 per cent. of the animals occurred in litters of three. Fifteen and fifty-five hundredths per cent. of the animals were born in litters of only one young, and 13.33 per cent. in litters of four individuals.

The next space below in the table shows the number and percentage of individuals living over three months

TABLE II

THE SIZE OF LITTERS AND MORTALITY OF DESCENDANTS FROM DIFFERENT COMBINATIONS

	Normal Lines 90				Alcoholic Lines 401				Normal Inbred 30				Alcoholic Inbred 135			
	1 in litt.	2 in litt.	3 in litt.	4 in litt.	1 in litt.	2 in litt.	3 in litt.	4 in litt.	1 in litt.	2 in litt.	3 in litt.	4 in litt.	1 in litt.	2 in litt.	3 in litt.	4 in litt.
Total number.	14 (15.55%)	46 (51.11%)	18 (20%)	12 (13.33%)	84 (20.94%)	214 (53.36%)	87 (21.69%)	16 (3.99%)	12 (40%)	18 (60%)	0	0	38 (28.14%)	76 (56.29%)	21 (15.55%)	0
	(Average number of young per litter 1.96)				(Average number of young per litter 1.79)				(Average number of young per litter 1.43)				(Average number of young per litter 1.62)			
Lived over 3 months....	12 (85.71%)	40 (86.95%)	11 (61.11%)	4 (33.33%)	54 (64.28%)	109 (50.93%)	19 (21.83%)	3 (18.75%)	12 (100%)	12 (66.66%)	0	0	29 (76.31%)	20 (26.31%)	0	0
	(All together 74.44%)				(All together 46.13%)				(All together 80%)				(All together 36.29%)			
Died within 3 months....	2 (14.28%)	6 (13.04%)	7 (38.88%)	8 (66.66%)	30 (35.71%)	105 (49.06%)	68 (78.16%)	13 (81.24%	0	6 (33.33%)	0	0	9 (23.68%)	56 (73.68%)	21 (100%)	0
	(All together 25.56%)				(All together 53.86%)				(All together 20%)				(All together 63.70%)			
Deformed....	0	0	0	0	2 (2.25%)	9 (4.20%)	10 (11.49%)	0	0	0	0	0	2 (5.26%)	14 (18.42%)	4 (19.04%)	0
					(All together 5.23%)								(All together 14.81%)			
Undersize (less than ⅔ of the average weight)....	0	0	0	0	1 (1.19%)	4 (1.86%)	3 (3.44%)	2 (12.50%)	0	1 (5.55%)	0	0	1 (2.89%)	4 (5.26%)	0	0
					(All together 2.49%)				(All together 3.33%)				(All together 3.70%)			

in the different-size litters. Almost 86 per cent. of the individuals born one in a litter lived, and about 87 per cent., 40 out of 46, of those born two in a litter lived. When there were three in a litter, however, only 61 per cent. lived and of those born four in a litter, it happened that only one third of them survived, though there were only a few in all. Of the total number of young from normal non-related parents 74.44 per cent. lived. Judging from these statistics litters of one or two young are the most vigorous and individuals born in litters of three or four are not so likely to be strong and long-lived.

The next space below gives the mortality records, which, of course, is merely another way of bringing out the above statements. The space following contains the number of deformed animals, but from the normal matings not one such individual has been produced. The last space gives the number of small-size or dwarf specimens also, none of which occur among these litters from normal non-related parents.

The second vertical column contains a similar analysis of the influence of the size of the litter on the mortality and condition of the young born from non-related alcoholic parentage. This not only includes the offspring from directly treated animals, but also other matings of non-relatives belonging to the alcoholic lines. Here again the majority of all the young, 53.36 per cent., are born in litters of two. Litters containing three are next in frequency, followed by litters of only a single individual. Of the total number of offspring produced by alcoholic parents 21.69 per cent. occurred in litters of three, and only about 4 per cent. of the offspring were members of litters of four individuals. The average number of young in the litters from these animals is 1.79, somewhat smaller than from normal matings.

The space below shows that in all only 46.13 per cent. of these young survived, whereas more than half as many more, or 74.44 per cent., from normal parentage lived over three months. The most vigorous animals are those

born only one in a litter. Sixty-four and twenty-eight hundredths per cent. of them lived. While about 51 per cent. of the two-in-a-litter individuals survived, only about 22 per cent. of the young born three in a litter were capable of surviving, and only 18.75 per cent. of the individuals from the litters of four lived more than three months. These figures indicate that the offspring from similarly injured parents are more capable of survival when born in a small litter of one or two than when contained in larger litters of three or four.

This is not on account of the fact that the treated or degenerate mother is more incapable of nourishing the larger litters, since the same is true of the larger litters from normal mothers, as shown by the previous column. The fact is that all young of large litters tend to be small and weak at birth, whereas a single young is far better accommodated. For these reasons it is always of importance to know the size of the litter in which an animal was born in estimating the degenerate qualities it may possess as compared with the qualities of another individual. For example, one animal may appear larger and stronger than another, and yet when bred will give rise to more degenerate offspring than the weaker individual. Although having a vigorous body, its germ-cell complex was not so good as that of the weaker animal, from a larger litter which produces better offspring. Therefore, the small weak males bred to normal females do not always give the poorer results when compared with the matings of stronger males and normal females.

The next space is the reverse of the one above and shows the percentages of mortality among the offspring derived from alcoholic non-relatives. More than half of the young, 53.86 per cent., from these combinations die soon after birth, a mortality record just twice as high as that of the control animals.

The next space shows the frequency of deformities among such young. Here it is again clearly indicated that the animals born one in a litter are better than those

FIG. 1. 521 albino F_2 ♀ (two alcoholic grandmothers, both grandfathers normal). Lived only one day after birth; the meninges of the brain were filled with blood. Gross tremor and complete paralysis of right side. Cataracts, both crystalline lenses being entirely opaque. The photograph shows the outstretched paralyzed right extremities while the left legs are held in a normal position in their effort to support the body. (Birth weight, 54 grams.)

FIG. 2. 506 mouse and yellow $F_{2,3}$ ♂ (two paternal great-grandmothers and the maternal grandmother alcoholic, slightly inbred). Gross tremor and complete paralysis of left side so unable to walk. Cornea of right eye opaque. Photograph shows the powerless condition of the outstretched left legs with the right legs attempting to support the body. (Birth weight, 57 grams.) The two figures are at different magnifications.

from litters of two, which are in turn better than the members of the litters of three individuals. Only 2.25 per cent. of the 84 individuals born in litters of one were deformed. While 9 of the 214 individuals born two in a litter, or 4.2 per cent., almost twice as many, were deformed. And 10 of the 87 animals born three in a litter were deformed, or about 11.5 per cent., which is almost five times higher than the number of deformities found among the animals born in litters of single individuals. Among the descendants of alcoholic non-relatives there was in all 5.23 per cent. of deformed specimens, whereas not one deformed animal arose from similar normal matings.

The last space of this column indicates the number of dwarf or undersize animals produced in the different litters from non-related alcoholic lines. Among the 84 animals born one in a litter only a single individual was of unusually small size. The 214 animals born in litters of two were all of average size except four, or stated exactly, 1.86 per cent. of them were undersize. In the litters of three 3.44 per cent. of the animals were small, while 12.5 per cent. of the members of litters of four were small specimens. Here again it is shown that the members of large litters are not so uniformly up to the standard of size and vigor as animals born in litters of only one or two individuals.

The third vertical column gives a similar analysis of the few normal inbred individuals which have been produced during the time of the experiment. There are not many such matings, as a general effort has been made to avoid inbreeding the control animals since this might be considered to vitiate the results.

The few young produced by inbred normal matings have all been in litters of only one or two offspring, so that the size of the litters averages only 1.43 individuals. The size of the litters is, therefore, smaller than from either the non-related normal or alcoholic animals. Eighty per cent. of the young have survived, more, however, from the

FIG. 3. 701 agouti, yellow and white, normal F_3 ♂. A normal animal from the fourth generation of the control; slightly inbred, natural size. Birth weight, 63 grams.)

FIG. 4. 599 black, white and red. F_3 ♂. A degenerate animal from the fourth generation alcoholic lines; no inbreeding; the paternal grandmother had both parents alcoholic and the maternal grandfather had both parents alcoholic. So there were two alcoholic great-grandmothers and two alcoholic great-grandfathers; the other four great-grandparents were normal and one grandmother and one grandfather had no alcoholism in their ancestry. The parents were ♂ (NN) (AA) ♀ (AA) (NN). The animal lived seven days and died in convulsions. The photograph shows the front limbs bent under the body and the animal is unable to raise the head. It weighed only 35 grams at the time of death, having lost 7 grams. While the above normal animal weighed 63 grams, actually a little small, at birth and all normal animals increase in weight rapidly from that time.

litters of one than from the litters of two. Not one deformed animal has resulted from these normal inbred matings and only one individual of the thirty was less than two thirds the average weight. The few normal inbred young here considered are then equally as good as the young from normal non-relatives and necessarily superior to the alcoholic lines. Judging from the results of others, there is little doubt that a more extensive inbreeding might produce deleterious effects.

The last vertical column indicates the effects of inbreeding alcoholic animals and their descendants. This combination shows the poorest quality offspring found in the table. Here again the members of the larger litters are at a disadvantage when compared with those born in the small litters. The average number of young in a litter is 1.62, somewhat smaller than the litters produced by mating alcoholic non-relatives. Thirty-eight litters contained a single individual each, the same number of litters contained two individuals, while only seven litters consisted of three young, and these were the largest litters produced. The inbred alcoholic animals, therefore, have a tendency to produce a large proportion of small litters and this tendency aids in strengthening their offspring.

Of the 135 young resulting from these matings, only 36.29 per cent. of them survived; this is the poorest life record shown by any combination. Of those born only one in a litter, however, 76.31 per cent. survived, which is a record equal to the average of the control. Therefore, even in this very bad combination, alcoholic inbreeding, when only one young is produced at a litter by an animal ordinarily capable of producing two or more, this one young is so well nourished and accommodated that it is somatically vigorous. Yet on breeding such individuals it almost always happens that very inferior offspring result. The germ cells, at any rate, may possibly be stronger than those in the weaker individuals which occurred in litters of two or three. Only 26.31 per cent. of the animals born in litters of two were capable of sur-

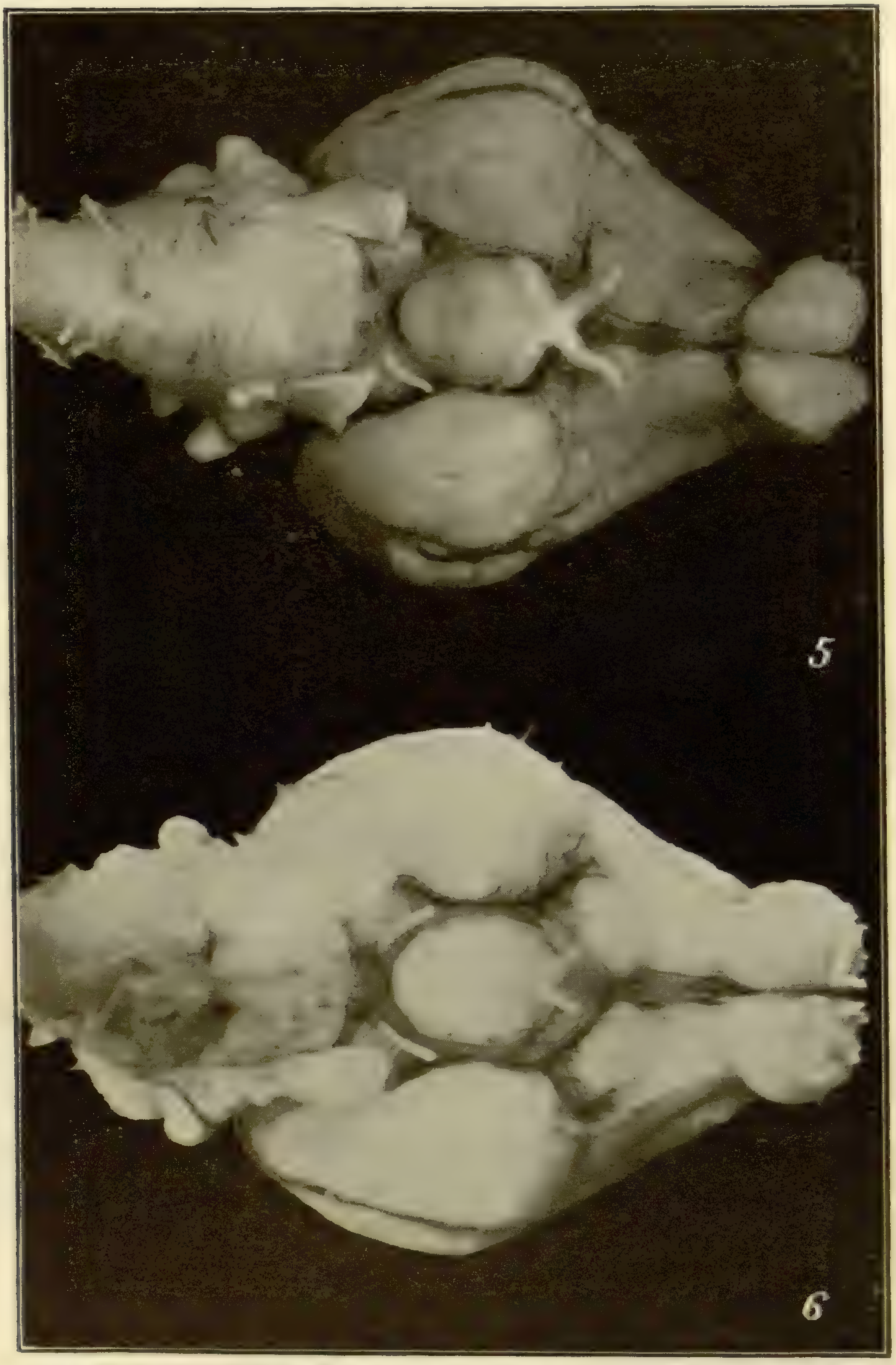


FIG. 5. Ventral view of a normal guinea-pig brain killed three days after birth. The optic nerves, optic chiasma and optic tracts passing along the tuber cinereum are distinctly seen.

FIG. 6. Ventral view of the brain of an anophthalmic monster from alcoholic great-grandfathers. There are no optic nerves, chiasma or tracts; the only indication of optic parts being two small membranous processes about in the position of the optic chiasma.

viving. The mortality here happens to be about three times higher than among the single-litter individuals. And further, not one of the 21 specimens born in litters of three lived. Among the offspring from the alcoholic inbred lines, judging from the numbers now available, the difference between the vitality of individuals born in litters of one and those born in larger-size litters is most striking.

The space below is the reverse of the one just considered and gives the percentage of young dying in the different litters. Only 23.68 per cent. died from litters of one individual, while 73.68 per cent. died in the litters of two individuals, and every one, 100 per cent., of the animals born in litters of three died within three months and usually within a few days.

The proportion of deformed animals occurring in the different-size litters again emphasizes the same differences in quality. All together 14.81 per cent. of the 135 individuals were grossly deformed; this is the highest percentage of deformed animals occurring in the several combinations represented in the table.

Of those animals born one in a litter only 5.26 per cent. were deformed; of those born two in a litter 18.42 per cent., or more than three times as many, were deformed, and of those specimens born in litters of three 19.04 per cent., or about one in five of them, were grossly deformed.

The proportion of deformities, therefore, conforms to the mortality records, being very much higher in the larger litters, and not unusually high among the individuals born one in a litter as compared with the average percentage of deformities from alcoholic non-relatives. Therefore, the bodily quality of the offspring is not materially worse from alcoholic inbred animals than from matings of alcoholic non-relatives, provided only one individual is born in the litter. But when more than one individual occurs in the litter, the alcoholic inbred combination is the most disastrous for the vitality and form of the offspring of all the combinations considered.

The last space shows that 3.7 per cent. of these offspring were less than two thirds the normal size. This again compares unfavorably with the other combinations, and here also the individuals born one in a litter show a superiority over those born in litters of two.

From a consideration of this table it may be concluded that the vigor of a guinea pig varies inversely with the size of the litter in which the animal is produced, and this is equally true whether the animal is born from normal or alcoholic parentage. However, the differences between the mortality of animals born in litters of one, two or three from normal parentage are not nearly so great as comparable differences between the members of the small and large litters from alcoholic lineage. For example, the difference in mortality between normal animals in litters of one or two is about 1 per cent., or scarcely any; between these and the mortality of specimens born three in a litter there is a difference in mortality record of about 24 per cent., to the discredit of the larger litters.

The comparable differences in the alcoholic lines is ever so much greater. There is almost 14 per cent. higher mortality among individuals from litters of two than from litters of one, and actually about 43 per cent. higher mortality among animals from litters of three than from litters of one. The difference between the mortality percentages in the litters of one and the litters of two from alcoholic inbred animals is 50 per cent. In other words, the mortality is three times as high among individuals from litters of two as from litters of one in inbred alcoholics, while the normal individuals born in litters of two are equally as good as those in litters of one. The parents from the injured alcoholic lines are incapable of producing large litters of strong individuals. The subnormal fetus fares pretty well alone in the uterus but is put at a great disadvantage by having to divide its uterine nourishment with brothers and sisters.

Another almost equally plausible explanation of this

striking difference in quality and vitality among the members of small and large litters might be given. It may be supposed that the growth capacity of the eggs maturing in the ovaries of normal and subnormal individuals depends somewhat upon the number of eggs maturing at any one time, or ovulation period. A normal animal may be capable of developing two entirely good eggs at an ovulation, or possibly three, whereas a weakened, less vigorous individual has ovaries incapable of producing more than one well-nourished or well-developed egg at any one time. Of course, it is understood that the small size of a mammalian egg would make it seem as though it required very little stored food from the ovary, yet that little must be of an extremely fine quality, since so much of the energy of early development is derived from the materials stored within the egg.

One point which might be interpreted to favor such an explanation is the fact that the small, weak young contained in the large litters do not recover and make their shortage good after birth, as might be expected if their inferior condition was simply due to a lack of nourishment available in the overcrowded uterine environment in which their late stages of development were passed. Lack of intra-ovarian nutrition would certainly produce a more lasting effect, since it occurs at an earlier stage than lack of uterine nutrition, though of course we do not pretend to deny that poor uterine nutrition would also leave its persisting mark.

When only one young was produced in a litter the average growth rate of such individuals during the first month after birth was 85.09 grams. Such specimens were not only largest at birth, but they grew fastest after birth. Animals born in litters of two increased 68.46 grams during the first month after birth, while those born three in a litter gained only an average of 63.6 grams during the same period. In other words, the last group only gained 75 per cent. of the amount gained by similar specimens which were fortunate enough to be developed alone in the ovary and in the uterus.

A second conclusion indicated by Table II is that inbreeding the defective alcoholic stock produces a quality of offspring decidedly inferior to that produced by the alcoholic lines when not inbred. This involves the internal factors of the germ cells. When a modified germ cell is united with a related one probably modified in a closely similar manner, a summation of the modification produces a more decidedly modified individual than would result from the combination of two non-related germ cells, even though they also be modified. In other words, as is shown in much of the data on heredity in higher animals, relatives probably respond to the treatment more nearly in the same way than do non-relatives, and there fore inbred defectives produce the most disastrous results obtainable.

The Relative Conditions of the Male and Female Descendants from Paternal and from Maternal Alcoholized Ancestors

We may now consider the possibility of analyzing the relative influences of various alcoholized ancestors on their offspring of different sex and the descendants of such offspring. The problems may be stated thus: are the offspring from alcoholized males more or less degenerate or modified than those from alcoholized females, and is there a difference in the degree of degeneracy between the male and female offspring? Are the descendants from alcoholic grandparents on the father's side more or less defective than the descendants from alcoholic grandparents on the mother's side, and do alcoholized grandfathers and grandmothers show an equally strong tendency to stamp their grandchildren? Do the grandsons and granddaughters show relatively different conditions, depending upon whether they are descended from alcoholized grandfathers on the father's or the mother's side or from alcoholized grandmothers on the paternal or the maternal side?

Table III, which excludes all inbred animals, is a sum-

TABLE III

THE RELATIVE DEGENERATIVE INFLUENCE OF MALE AND FEMALE ALCOHOLIZED ANCESTORS ON THEIR MALE AND FEMALE DESCENDANTS. (ALL INBRED ANIMALS ARE EXCLUDED)

	Males with	Females with	Young of Unknown Sex with	Males with	Females with	Young of Unknown Sex with	Males with	Females with	Young of Unknown Sex with	Males with	Females with	Young of Unknown Sex with	Males with	Females with	Young of Unknown Sex with	Males with	Females with	Young of Unknown Sex with
	Alcoholized Father			Alcoholized Mother			Alcoholized Grandfather on the Father's Side			Alcoholized Grandfather on the Mother's Side			Alcoholized Grandmother on the Father's Side			Alcoholized Grandmother on the Mother's Side		
Total number..	44	43	70	37	23	38	36	38	26	34	32	48	37	33	36	23	18	25
Lived over 3 months.....	37 84.1%	33 76.74%	0	34 91.89%	22 95.65%	0	26 72.22%	23 60.52%	0	23 67.64%	19 59.37%	0	25 67.56%	23 69.69%	0	18 78.25%	14 77.77%	0
	44.58%			57.14%			49%			36.84%			45.28%			48.48%		
Died within 3 months......	7 15.9%	10 23.25%	70 100%	3 8.1%	1 4.34%	38 100%	10 27.77%	15 39.47%	26 100%	11 32.35%	13 40.62%	48 100%	12 32.43%	10 30.30%	36 100%	5 21.74%	4 22.22%	25 100%
	55.41%			42.85%			51.0%			63.15%			54.71%			51.51%		
Deformed.....	1 2.27%	2 4.65%	3 4.28%	0	0	0	3 8.33%	4 10.52%	0	1 2.64%	4 12.5%	7 14.58%	2 5.40%	3 9.03%	7 16.66%	2 8.69%	0	0
	3.82%			0			7.0%			10.52%			10.37%			2.77%		

marized analysis of these questions. The male and female descendants from six different lines are tabulated. The table is not perfectly pure, but merely represents a mass result, since, for instance, in giving the young from alcoholized fathers some of these young had also alcoholized grandparents, etc. The same is true of the other lines. But the large majority of the figures are from unmixed matings, so that these mass results do have some real significance.

In the first vertical section is given the records of offspring from alcoholized fathers. Forty-four males, 43 females and 70 young of unknown sex are considered. Of the males 84 per cent. lived, and 76.7 per cent. of the females lived. These numbers are very high, since in the early part of the experiment only those young which survived were catalogued for sex. Therefore, all of the 70 young of unknown sex were animals which died at birth or soon after, and as the table shows more than half of the animals from alcoholized fathers died soon after birth.

The mortality among the male offspring from alcoholized fathers was 15.9 per cent., while among the female offspring it was considerably higher, being 23.25 per cent. The same difference in quality between the sexes is illustrated by the percentage of gross deformities. Only 2.27 per cent. of the males were deformed, while twice as great a proportion, or 4.65 per cent., of the female offspring from treated fathers were deformed. In all 3.82 per cent. of the offspring from alcoholized males were deformed and the female offspring were inferior in quality to the male.

The next section of the table presents similar data for the offspring from alcoholized mothers. There were 37 male, 23 female and 38 offspring of unknown sex. Again, the offspring in which the sex was ascertained during part of the experiment were only those that survived, therefore, their mortality record is very good, while all the animals of unknown sex were individuals that died shortly after birth. Yet the records of the males and fe-

males are based on exactly the same kind of data and are to be fully compared. Eight and one tenth per cent. of the males died, while only 4.34 per cent., proportionately about half as many, females died. Not one grossly deformed animal was found among the offspring of alcoholized females.

Thus from the mortality records the sons of alcoholized mothers appear more affected than their daughters. And taken as a whole the records of the alcoholized mothers are superior in quality to those of the alcoholized fathers, thus indicating that the male germ cells are more injured by the treatment than the female germ cells.

The third section shows the records of the male and female descendants from alcoholized grandfathers on the father's side. Here the mortality record of the males is much better than that of the females; 27.77 per cent. of the males died soon after birth, and 39.47 per cent., a very much higher proportion of the females, died.

The mortality of these animals from alcoholic grandparents seems much greater than that of animals from treated parents; this is due, however, to the fact that the sex of many more of these that died at birth was ascertained as they occurred later in the experiment when this point was being watched. The totals are the only figures in the horizontal mortality columns that are to be compared. The total mortality of descendants from alcoholic grandfathers on the father's side was 51 per cent., which is higher than the mortality of the offspring from alcoholized mothers, 42.85 per cent., but lower than the mortality of offspring from alcoholized fathers, which reached 55.41 per cent.

Among the ascertained male descendants from an alcoholized paternal grandfather 8.33 per cent. showed gross deformities, while 10.52 per cent. of the descendants ascertained to be female were deformed. Considering all the animals in this group, 7 per cent. were deformed, which is almost twice as great a proportion as occurred among the offspring of alcoholized fathers. *The deform-*

ities in the F_2 generation are more frequent than in the F_1.

The fourth section shows the influence on the grandchildren of an alcoholized grandfather on the mother's side. This is the most injurious combination shown. Only 36.84 per cent. of the offspring survive. Of the male descendants of an alcoholized maternal grandfather 32.35 per cent. died soon after birth, while proportionally many more, or 40.62 per cent., of the female descendants died. In all a total of 63.15 per cent. of the descendants from alcoholized maternal grandfathers died, which is the highest mortality record obtained.

Among the grandchildren of alcoholized maternal grandfathers 10.52 per cent. were deformed, a very high proportion. But of the grandsons only 2.64 per cent. were deformed, while almost five times as many, or 12.5 per cent., of the granddaughters were grossly deformed. Thus *the females of the F_2 generation from a treated maternal grandfather are poorer when considered from the standpoint of mortality record and bodily structure than the male F_2's from the same source.*

The fifth line to be considered is that of an alcoholized grandmother on the father's side. The result of this treatment as shown by the grandchildren is very bad, but not quite so bad as from the alcoholized maternal grandfather just discussed.

From alcoholized paternal grandmothers the conditions of 37 grandsons and 33 granddaughters are to be compared. About the same survival record is shown by both sexes: 67.56 per cent. of the male grandchildren lived and 69.69 per cent. of the females lived. Of all the descendants from this combination, including those in which the sex was not determined, only 45.28 per cent. survived, giving a mortality record of 54.71 per cent., considerably better, by almost 10 per cent., than that of animals from an alcoholized maternal grandfather in the preceding section.

A large proportion of the animals from alcoholized paternal grandmothers were deformed, 10.37 per cent. However, only 5.4 per cent. of the grandsons were de-

formed, while many more, 9.03 per cent., of the granddaughters were deformed and among the young of undetermined sex 16.66 per cent. were deformed.

In the last section the records of descendants from alcoholized grandmothers on the mother's side are given. There were 23 males, 18 females, and 25 young which died with their sex unascertained. Forty-eight and forty-eight hundredths per cent. of the animals lived. The mortality among the males was 21.74 per cent., about the same as that of the females which was 22.22 per cent. The total mortality being 51.51 per cent. From this combination there occurred a low percentage of deformities, confined entirely to the grandsons. *So that 8.69 per cent. of the grandsons from alcoholized maternal grandmothers were deformed, while none of the granddaughters showed any gross structural abnormalities.*

TABLE IV

MORTALITY DURING THE FIRST THREE MONTHS OF THE DESCENDANTS OF KNOWN SEX FROM ALCOHOLIC ANIMALS (NOT INBRED)

Treated with Alcohol	Males			Females			All Together		
	Total Number	Died Early	Mortality	Total Number	Died Early	Mortality	Total Number	Died Early	Mortality
Father	44	7	15.90%	43	10	23.25%	87	17	*19.54%*
Mother	37	3	8.10%	23	1	4.34%	60	4	*6.66%*
Grandfather on father's side	36	10	27.77%	38	15	39.47%	74	25	*33.78%*
Grandmother on father's side	37	12	32.43%	33	10	30.30%	70	22	*31.42%*
Grandfather on mother's side	34	11	32.35%	32	13	40.62%	66	24	*36.36%*
Grandmother on mother's side	23	5	21.74%	18	4	22.22%	41	9	*21.95%*

Table IV presents in a more concise manner certain of the figures considered in the foregoing Table III. Only the mortality records of the male and female descendants from different sources and the total mortality of the several groups is shown by the table and thus a ready comparison of the conditions may be made. Among the offspring from alcoholic fathers 15.9 per cent. of the males died and 23.25 per cent. of the females. *The fe-*

male are more injured than the male offspring of treated fathers. The next horizontal line shows that *the offspring from treated mothers are far better than from treated fathers,* having a much lower mortality. *The male germ cell is more affected by the alcohol than the ovum, therefore treated fathers produce poorer offspring than treated mothers.*

The heterogeneous female descendants from an alcoholized paternal grandfather are more affected than the male, 39.47 per cent. mortality to 27.77 per cent.

The male and female descendants from an alcoholized paternal grandmother show about equal conditions, 32.43 per cent. male mortality to 30.3 per cent. female mortality.

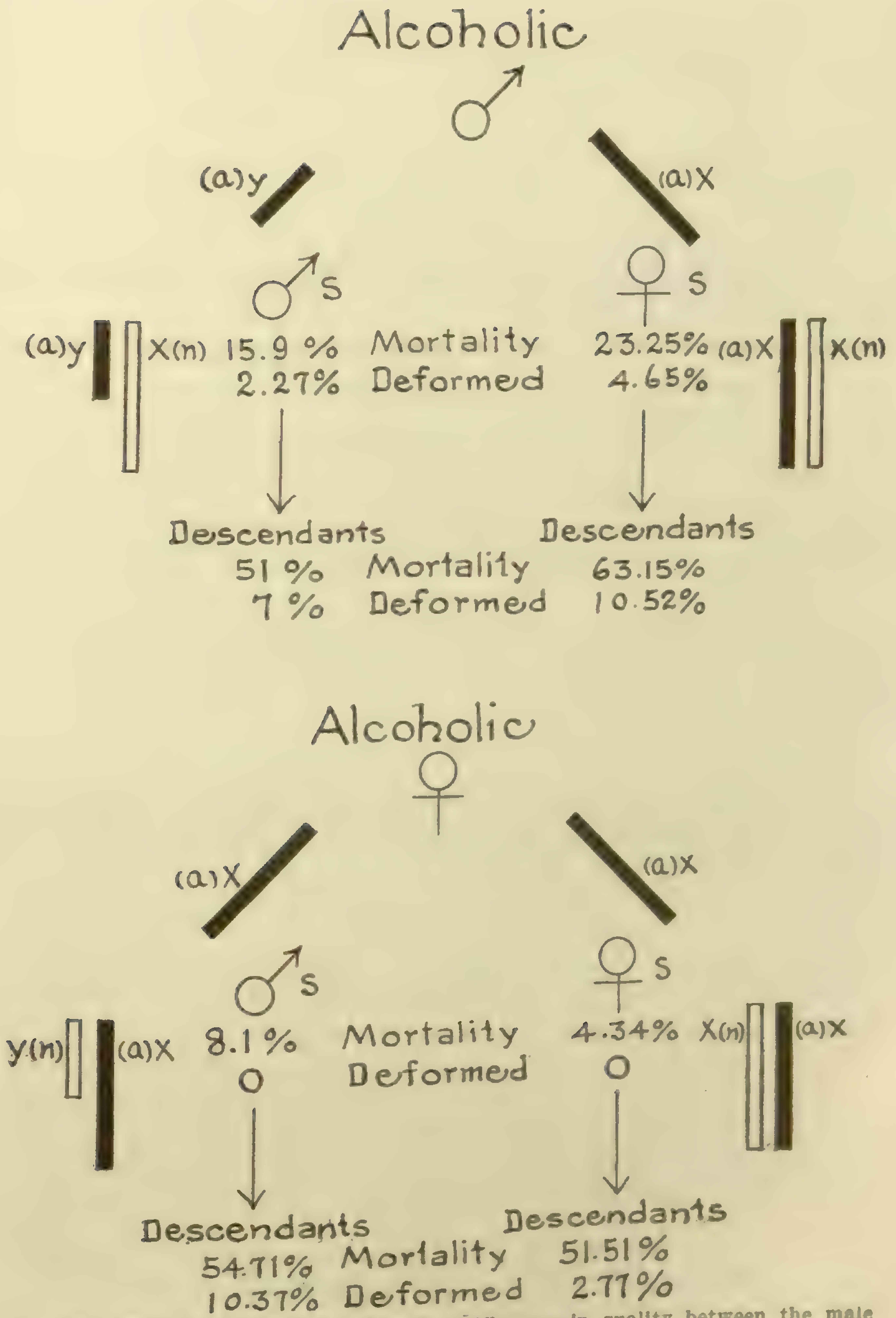
The heterogeneous female descendants are inferior to similar male descendants from an alcoholized maternal grandfather, 40.72 per cent. female mortality to 32.25 per cent. male mortality.

The male and female descendants are about equally strong from an alcoholized maternal grandmother, 21.74 per cent. male mortality to 22.22 per cent. female mortality.

Although explanations of the above differences between the ways in which the male and female guinea pigs are affected by the treatment, as well as explanations of the different records of the grandsons and granddaughters from alcoholization of different ones of the four grandparents, are difficult to give at the present stage of the experiment, a tentative explanation based on the composition of the chromosomal complex is certainly suggested.

General Considerations

In the case of the male guinea pig, according to the studies of Miss Stevens ('11), two kinds of spermatozoa are produced. The one has a large X chromosome, the female-producing spermatozoon, and the other contains a corresponding small Y chromosome, which from homology with other forms we may consider to be the male-producing. The two classes of spermatids are different



The diagram intended to show the difference in quality between the male and female offspring of alcoholized males and females, and indicating a possible explanation of these differences. The upper part of the diagram represents the heteromorphic chromosomes X and Y of the male passing to the male and female offspring. The larger injured X chromosome gives a larger proportion of deformities and a higher mortality to the females and their descendants. The smaller injured y chromosome gives to the males and their descendants a proportionately smaller amount of deformities and a lower mortality record.

The lower part of the diagram shows equal amounts of injured chromatin passing from the treated mother to her male and female offspring, the black X chromosomes. But these equal amounts of modified chromat'n are combined with unequal amounts of normal chromatin from the heteromorphic spermatozoa, the unequal white X and Y chromosomes. The male offspring, therefore, have in their chromosomal constitution proportionally more modified and less normal chromatin than the female offspring which have equal amounts of normal and alcoholic. And the male offspring and their descendants are actually found to show a higher mortality and a greater proportion of deformities than the female offspring and their descendants.

morphologically and we may expect them to differ in their susceptibilities to the alcoholic treatment. One class may be more affected than the other. This might be due simply to the reason that one class of spermatozoa actually according to mass has more chromatin to be acted upon than the other. And this difference in mass of affected chromatin might be sufficient to give a difference in quality between the individuals arising from the two classes of spermatozoa.

At any rate, as the accompanying diagram indicates, there is a decided difference between the records of the male and female offspring from treated guinea pigs. The upper half of the accompanying diagram shows that the mortality is higher and the gross deformities more frequent among the female offspring sired by alcoholized male guinea pigs than among the male offspring. This difference we may venture to suppose is due to the fact that the female offspring actually receive more modified or injured chromatin from the alcoholic father than do the sons. The diagram is an attempt to represent this larger mass of injured chromatin, the large black X chromosome passing to the daughters, while the smaller black Y chromosome is received by the sons.

Another possible explanation might be that the two heteromorphic sex chromosomes, the X and Y, respond differently to the influence of the alcoholic treatment, the X being the more affected. Such an opinion has some basis, since these chromosomes in the later development of the two sexes seem to carry such a number of contrasting qualities according to the splendid evidence presented by Morgan and his associates. One may be permitted to assume on probability, at any rate, that the X and Y chromosomes are qualitatively different in their finer chemical constitutions, and this qualitative difference would necessitate a different response to the chemical treatment on the part of each of the two chromosomes.

There is also important evidence from the parthenogenetic groups, as, for example, the Phylloxerans and

Aphids (Morgan, '09), which might lead one to believe that the two classes of spermatids or finally spermatozoa are never quite equally active or vigorous. This difference may vary from apparent equality in most higher animals to cases such as the parthenogenetic Phylloxerans and Aphids, in which one class of spermatids are actually degenerate and non-functional.

In this connection an experiment performed with a quite different problem in view by Cole and Davis ('14) with alcoholized rabbits is suggestive. They found that when two male rabbits were mated with a female superfetation occurred in most cases so that part of the resulting litter of young were sired by one male and part by the other. The two males differed in their ability, so that one more often sired the majority of young of a given litter and in the total number of competition matings sired the greater number of young. When this male with the fertilizing advantage was treated for a short period of time, a month or more, with fumes of alcohol he was then affected in such a way that when mated in competition with the same male he normally had beaten he now failed to sire any young. Yet if mated singly or alone with a female he still had the power to beget offspring. The alcohol treatment had in some way lowered the power of his spermatozoa to fertilize an egg. Thus these spermatozoa could no longer fertilize an egg in the presence of the spermatozoa from a male which was originally less potent than they.

All of these data indicate differences in the behavior and reactions of the individual germ cells, and such differences probably account for the discrepancy existing between the conditions of the male and female offspring from an alcoholized father. Since this point has only recently been discovered in the experiments, we now have very few definite matings to test its meaning by back crosses with the normal. But a large number of heterogeneous matings have been made during the last few years and their gross results serve to verify the fact that

the difference in quality between male and female offspring is actual, although such matings furnish no definite analysis of the conditions.

In the first place, the upper half of the diagram shows that the mortality is higher and the defects more frequent among the female offspring of treated males than among their sons. The products of the heterogeneous matings in which these male and female offspring have taken part go to indicate that the first apparent difference in their records was a real difference. The mortality record of the mass descendants from the sons of alcoholized fathers is about 20 per cent. better than the mortality record from the descendants of the daughters of alcoholic fathers. And the proportion of deformities is 50 per cent. higher among the descendants of the daughters than among the descendants of the sons. These conditions of the descendants prove that the female offspring from the alcoholized males are actually worse than the male offspring in the following respects: their mortality record, the frequency of deformities, and the quality of young to which they give rise. The only plausible way to account for the origin of this difference is to assume that the female-producing spermatozoa were more modified by the treatment than the male-producing spermatozoa. Whether such an increased modification is due to the presence of a greater mass of chromatin to be injured in the one case than in the other or to a difference in response on the part of the two heteromorphic sex chromosomes it is impossible to state. The difference, however, is a fact!

The lower half of the diagram illustrates the different qualities of the male and female offspring from alcoholized mothers. Here each sex of the offspring in accordance with prevalent cytological views receives an equal amount of chromatin from the treated mother. And, moreover, as far as the treated mother is concerned similar chromosomal complexes are conveyed to both sexes of the offspring. The two classes of young should, therefore, show similar conditions, but such is not the case.

The mortality of the male offspring is higher than that of the female. This condition may probably be explained on the same principles we have employed above. The two sexes receive equal amounts of injured chromatin from their alcoholized mother, but this injured chromatin in the case of the female individuals is mixed with a larger amount of normal chromatin from the father than is the case with the male. The female combination of equal amounts of good and bad chromatin gives rise to a better product than the male combination of a larger amount of modified chromatin with a smaller amount of good. Therefore the records of the male offspring are inferior to those of the female offspring.

The female combination, ovum and spermatozoon with equal amounts of chromatin, good and bad, is proportionately less injured than the male combination, ovum with a larger amount of bad and spermatozoon with a smaller amount of normal chromatin. The diagram represents the black X chromosomes equal in size passing to the daughters and the sons to be combined with the large white normal X in the case of the daughters and with the small white normal Y in the case of the sons.

Again the descendants from heterogeneous matings of these males and females prove that there is an actual difference in quality. The descendants from the sons of alcoholic mothers show a slightly higher mortality and a much greater proportion of deformities than are found among the descendants of their daughters.

We believe that these results actually show a difference in response to the treatment on the part of the male- and female-producing spermatozoa. Such a difference logically follows the cytological differences in structure which Wilson and others have so clearly demonstrated during the past ten years. If this structural difference is of any significance, as it surely must be, then such physiological differences in behavior as are indicated in our results should sooner or later be found.

On such a basis as this the sex ratio in different classes

of animals may possibly be explained. A species such as man, which constantly seems to produce more males than females, may be said to form more active or vigorous male-producing spermatozoa. In the competition to fertilize the egg such spermatozoa win an advantage and in the sum total more males than females arise, the ratio depending upon the extent of the advantage the one class of spermatozoa has over the other.

We now have under way a number of matings which are designed to test these propositions in an analytical fashion. One of us (Papanicolaou, '15) is in possession of data giving reason to believe that a second explanation may be offered to account for the different conditions presented by the male and female offspring produced by alcoholized females. Such an explanation is based on the supposition that the female guinea pig as well as the male has a share in the determination of the sex ratio and may produce two kinds of ova. Such an explanation in its final analysis is extremely complex and unnecessary in the present discussion, though it will be presented in a future consideration of the regulation of the sex ratio in these animals.

Admitting, as is suggested above, that the two groups of spermatozoa differ in their response and resistance to the treatment, we may also admit that there are other normal differences in their vitality and behavior. These normal differences must also vary within certain limits. In one group of animals the female-producing spermatozoa may be more active and possess a higher degree of fertilizing power than the male-producing spermatozoa. Such a group would show a sex ratio below one hundred, there being more females than males produced. In other species of animals with a sex ratio of more than 100 the reverse condition obtains; the male-producing spermatozoa possess on an average a higher fertilizing power than the female-producing. But the advantage of the male-producing sperm may be slight and no doubt many individual males tend to form female-producing sperm

with a higher fertilizing power than the male-producing. Such individuals will more frequently beget female offspring. Slight differences in the physiological behavior of the two classes of spermatozoa would account for the sex ratios in all animals, and finally, as Morgan has shown, the extreme difference between the qualities of the two classes of spermatozoa leads to the degeneration of one entire class and the necessary production of only one sex from the fertilized eggs of these species. Such species must also be parthenogenetic in order to produce individuals of the other sex.

This discovery by Morgan suggests, as Wilson ('11) brings out in his review of the sex chromosome question, a plausible explanation of the sex ratios in different classes of animals. And we believe the evidence presented above lends further support to such an interpretation.

A rather old popular idea in attempting to explain the sex riddle may have some ground of fact from the standpoint of the variations in the differences of fertilizing power of the two classes of spermatozoa. It has often been claimed that one testis is male-producing and the other female-producing. Every one knows that this is untrue. Yet one testis may have a tendency to produce spermatozoa of the female class with a higher fertilizing power than the male sperm of this testis, and the other testis might have an opposite tendency, since the conditions of behavior often differ in two organs of a bilateral pair. An animal which has produced a large proportion of male offspring may after semi-castration produce almost all female offspring. A possible explanation for such an occurrence would be that the removed testis had produced more vigorous male sperm than female and the spermatozoa of this testis possessed the higher fertilizing power, while the remaining testis tended to produce more potent female sperm. On removing the one testis the other came into supremacy. In the same imaginary case, if the opposite testis had been removed, there would have

been no change in the tendency to produce offspring of a certain sex, since the remaining testis originally possessed an advantage.

Finally, then, from the above experiments there is no question that the material basis of the hereditary qualities has been injured, since alcoholized males have transmitted the injury to four generations during a period of almost five years. In other words, as stated above, chromatin injured five years ago is now living in the great-grandchildren of the individuals in which it was injured.

Bardeen with the X-ray and Oscar Hertwig with radium have induced similar injuries by directly treating the spermatozoa, but these cells were so greatly injured that only the immediate effect upon the developing embryo was shown. The present experiments, however, demonstrate the passage or transmission of the injured chromatin from generation to generation during a period of years. *The behavior of the carriers of heredity becomes pathological just as any other organ with a normal function may behave in an abnormal or pathological manner.*

Mammals are particularly adapted to the study of such features of heredity as this, on account of their typical structure and large, easily observed organs. The complexity of their structure and behavior further permit the possibility of slight modifications becoming visible through abnormal conditions of their nervous system, etc. Thus with such material as guinea pigs a few experiments of this kind may furnish certain clues to the processes of behavior of the chromosomes that less plastic and simpler forms might never present in such a manner as would be recognizable.

On the other hand, the small litters and comparatively slow breeding render these higher animals unsuitable for an exhaustive analysis of many of the intricate problems of normal heredity.

Summary and Conclusions

In the foregoing pages we have considered the results of an experiment now in progress for more than five years which analyzes to some extent the influence on the offspring of alcoholizing either one or both parents and the manner of hereditary transmission of the induced effects to subsequent generations.

The experiments have demonstrated on two different stocks of normal guinea pigs that the parental germ cells may be so modified by chemical treatments that they are rendered incapable of giving rise to a perfectly normal offspring. This incapacity is probably due to modifications of the chromatin or carriers of the hereditary qualities within the germ cells, since the great-grandchildren, the F_3 generation, from the treated animals are usually more decidedly affected and injured than the immediate offspring (F_1) of the alcoholized animals.

This then becomes a study of the behavior of diseased or pathological chromatin in heredity. Chromatin rendered pathological more than four years ago is still living and has now been passed on to the F_3 generation from the alcoholized great-grandparents. The F_3 animals are almost without exception incapable of reproduction and are in many ways subnormal and degenerate.

Studies of abnormal heredity may possibly furnish a means of analyzing the normal methods of action by which the minute carriers of hereditary qualities contained within the fertilized egg are capable of causing complex developmental and structural changes to reoccur from generation to generation in so wonderfully consistent a manner. Just as the knowledge furnished by studies of experimentally modified embryonic development has supplied valuable data towards a clearer understanding of the normal processes and changes which occur in the developing embryo.

The treatment of adult guinea pigs by an inhalation method with daily doses of alcohol through several years produces little if any noticeable effect upon the organs

and tissues of the animal's body. The direct action of alcohol fumes tends to injure the respiratory mucosa and to render the cornea of the eye dull or opaque. These changes, however, do not inconvenience the animals in any perceptible way, and they remain strong and hardy and live as long and actively as the untreated guinea pigs. In spite of their healthy appearance the injurious influence of the alcohol inhalation is very decidedly shown by the quality of offspring to which the treated animals give rise. And the descendants of these offspring are even worse than the F_1 generation when compared with the different generations of control animals produced under identical cage and food conditions.

The males seem to be more injured by the treatment than the females, taking as an index of injury the quality of their offspring and descendants. Stating it differently, the spermatocytes or spermatozoa are more sensitive to the changed chemical condition of the tissues than are the female germ cells.

There is a larger proportion of degenerate, paralytic and grossly deformed individuals descended from the alcoholized males than from the alcoholized females.

The records of 682 offspring produced by 571 matings of animals of various types have been tabulated to show the kinds of litters of young produced and their ability to survive. One hundred and sixty-four matings of alcoholized animals, in which either the father, mother, or both were alcoholic, gave 64, or almost 40 per cent., negative results or early abortions, while only 25 per cent. of the control matings failed to give full-term litters. Of the 100 full-term litters from alcoholic parents 18 per cent. contained stillborn young, and only 50 per cent. of all the matings resulted in living litters. Forty-six per cent. of the individuals in the litters of living young died very soon after birth. In contrast to this record 73 per cent. of the 90 control matings gave living litters and 84 per cent. of the young in these litters survived as normal, healthy animals.

The mating records of the descendants of the alcoholized guinea pigs, although they themselves were not treated with alcohol, compare in some respects even more unfavorably with the control records than does the above data from the directly alcoholized animals.

Of 194 matings of F_1 animals in various combinations 55 have resulted in negative results or early abortions, 18 stillborn litters of 41 young occurred, and 17 per cent. of these stillborn young were deformed. One hundred and twenty-one living litters contained 199 young, but 94 of these died within a few days and almost 15 per cent. of them were deformed, while 105 survived and 7 of these showed eye deformities. Among 126 full-term control young of the same stock not one has been deformed.

The records of the matings of F_2 animals are still worse, higher mortality and more pronounced deformities, while the few F_3 individuals which have survived are generally weak and in many instances appear to be quite sterile even though paired with vigorous, prolific, normal mates.

The structural defects shown by the descendants of alcoholized animals seem to be confined chiefly to the central nervous system and special sense organs. Many of the young animals show gross tremors, paralysis agitans; the hind legs, fore legs or both legs of one side may be paralyzed (Plates I and II). Eye defects are very common, such as opaque cornea, opaque lens, various degrees of monophthalmicum asymmetricum, and finally several cases of complete anophthalmia have occurred, the entire eyeballs, optic nerves and optic chiasma being absent (Figs. 1 to 3 and Plate III).

The quality of individuals from the same parentage varies inversely with the size of the litters in which they are produced. Animals born one in a litter are rather strong, even though derived from very bad alcoholic lines. This difference between the members of small and large litters is also shown by the normal animals, but the difference in quality between members of large and small litters

is ever so much greater in the alcoholic lines. There is also some tendency on the part of the alcoholic animals to produce a greater proportion of small litters and this aids somewhat towards the perpetuation of their lines.

Inbreeding tends to emphasize the alcoholic effects. This is probably due to related animals responding to the treatment in closely similar ways on account of the similarity of their constitutions. Inbreeding, as such, may be harmful. But inbreeding added to the alcohol effects produces a much worse condition in the offspring than either inbreeding or alcoholism alone could do.

The data from alcoholized male lines indicate that the *female offspring from alcoholic males are less viable and more frequently deformed than the male offspring. And heterogeneous matings of such male and female offspring further emphasize the same inferiority on the part of the female offspring from treated males.* This is a very significant fact.

The fact that the offspring of one sex differ in quality from those of the opposite sex, and that the female offspring of an alcoholic male are inferior to his male offspring suggests at once a difference between the germ cells concerned in the production of the male and female young. Miss Stevens showed that the spermatocytes of the male guinea pig contained a heteromorphic pair of chromosomes and half of the spermatozoa would be expected to receive one member, the X chromosome, of the heteromorphic pair and one half of the spermatozoa the other member, the Y chromosome, of the heteromorphic pair. We now have two possibilities in explanation of the above facts. In the first place, it may be assumed that the alcohol acts similarly on all of the chromatin to injure it. Thus a mass action would cause the spermatozoa carrying the larger member of the heteromorphic pair to deliver more injured chromatin and the other spermatozoa with a less total amount of injured chromatin would deliver less when they fertilize eggs containing equal amounts of normal chromatin. The fertilized

egg giving rise to the female, therefore, contains a greater proportional amount of alcoholic chromatin to normal chromatin than does the egg giving rise to the male. And so the female product is actually more injured than the male.

A second possible explanation of these conditions may be that the X and Y chromosomes themselves respond differently to the treatment, the X being the more sensitive of the two. But in either case the two classes of spermatozoa certainly seem to respond differently to the treatment and this shows a physiological difference in behavior to correspond with the well-known morphological differences so often found between the two groups of spermatids of many animal species.

The data from alcoholic female lines indicates that *the male offspring from alcoholic females are inferior in quality to the female offspring. And heterogeneous matings of such male and female offspring further prove the inferiority on the part of the male offspring from treated mothers.* This is also significant. How can it be put in accord with the above chromosomal explanations for the difference in quality between the female and male young of alcoholized fathers?

If we admit that all of the eggs arising from an alcoholized female guinea pig are homomorphic and contain groups of chromosomes equal in mass, it follows that her male and female offspring receive the same amount of injured chromatin and should be affected by such chromatin to equal degrees. But this is only part of the case, the injured female chromatin is combined with normal chromatin from the normal father when the eggs are fertilized and here the difference arises. The female offspring receives from the normal father a larger amount of normal chromatin than do the male offspring. So that the female arises from an egg in which equal amounts of good and injured chromatin are present, while the male offspring arises from an egg in which a larger amount of injured chromatin is united with a smaller amount of

normal. Therefore, proportionally, the male offspring from treated mothers have more injured chromatin in their entire bodily make up than do the female offspring, and are comparatively in a more abnormal condition.

Another explanation of these differences between the male and female offspring of alcoholized females could be based on the possibility of the female being heterozygous for sex. This involves a very complex discussion, but one for which there is some ground on the basis of the regulation of the sex ratio in these animals.

Finally, then, the experiments show the hereditary transmission through several generations of conditions resulting from an artificially induced change in the germ cells of one generation. And they furnish data of importance bearing upon the pathological behavior of the carriers of heredity as well as the differences in behavior between the two types of germ cells produced by an animal carrying heteromorphic chromosomes.

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SHORTER ARTICLES AND DISCUSSION

VARIABILITY UNDER INBREEDING AND CROSS-BREEDING

An unusually thoughtful and suggestive discussion of evolutionary problems is contained in Professor Walton's paper on "variability and amphimixis" published in the November, 1915, number of this journal. But the paper is in some danger of neglect because the conclusions reached are apparently so revolutionary that to a casual reader they may seem freakish. Yet it will be seen by one who reads the paper more carefully that the radical character of its conclusions is due in part to the fact that certain familiar ideas are here viewed at a new angle. Nevertheless the new point of view has, it seems to me, to some extent, caused the author loss of perspective in relation to some of the phenomena which he discusses, for which reason further consideration of them may be profitable.

The occasion of Walton's discussion was a biometric study which he made of two sorts of zygospores produced by *Spirogyra inflata,* one sort produced by union of cells in the same filament (called by him "close fertilization"), the other by the union of cells in different filaments (called "cross fertilization"). Zygospores of the former sort ("close fertilized") were found to be on the average larger and more variable than those of the latter sort, *contrary to the prevailing idea that cross fertilization leads to increased variability.* It may however be questioned whether Walton's material is such as to throw new light on this question, for it is by no means certain that cells of *Spirogyra* which unite in lateral conjugation are the exact equivalents morphologically and physiologically of those which unite in scalariform conjugation. It is conceivable that zygospores formed in lateral conjugation may be larger and more variable because the cells which gave rise to them were as a group larger and more variable. It is conceivable that cells which resort to scalariform conjugation are not such as can satisfy their physiological demands for conjugation by uniting with a sister-cell in the same filament. For it is known that in many plants sexual union occurs only as a last resort, when conditions

are unfavorable for further continuance of the organism either by vegetative reproduction or even by parthenogenesis.[1] But whether or not Walton's own observations are considered pertinent, the question which it leads him to consider is one of profound evolutionary significance—does cross fertilization (as compared with close fertilization) tend to produce greater or less variability.

In parts of his discussion Walton fails to keep clearly in view distinctions, which he nevertheless recognizes, between the respective variabilities of F_1, F_2 and mixed populations. The fact has been known since the days of the early plant hybridizers, and is expressed clearly in one of Focke's laws of hybridization that the first generation (F_1) offspring of a hybrid cross are not, as a rule, more variable than the more variable parent race. In other words the generalization which Walton attacks, that crossing produces variability, is not commonly, if at all, held by biologists to apply to F_1 populations but only to the conditions obtaining in subsequent generations. But Walton's own observations are made exclusively upon F_1 zygotes. Supposing his two classes of zygotes to be morphological and physiological equivalents of each other (which, however, may reasonably be questioned) there was no ground for expecting one sort to be more variable than the other, so far as existing knowledge of the effects of inbreeding and cross-breeding is concerned.

Walton cites two experimental investigations, in support of his own observations on *Spirogyra,* to show that close fertilization produces greater variability than cross fertilization, viz., that of Jennings on *Paramecium* and that of Barrows and myself on *Drosophila.* But neither of these investigations deals with the same sort of cases as Walton's. Jennings is comparing the variability of conjugants with that of non-conjugants. This is a case where sexual is contrasted with asexual reproduction and is in no way comparable with a case in which the effects of cross and close fertilization are compared with each other. I quite agree with Walton's conclusion that the results are statistically considered far from conclusive, and would add that they are quite aside from the question which Walton is considering. Barrow's comparisons were made between *single lines* of *Drosophila* inbred (brother with sister) for from 30 to 61 generations and a culture derived from two original pairs of

[1] See Coulter, 1914, "The Evolution of Sex in Plants."

Drosophila the descendants of each pair being allowed to interbreed freely. As to the results we said (p. 776):

> These experiments show no appreciable effect of inbreeding (on variability). In every case the brood reared under the best and the most uniform conditions has the highest average number of teeth (in the sex-comb), irrespective of whether or not inbred. The same may be said of variation in size. Inbreeding has diminished neither the average size nor the variability in size.

Walton considers these conclusions justified by our statistical constants in the case of the sex-comb, but believes that a significant difference is observed in length of tibia, which we found to be both greater and less variable in the culture not inbred. He criticizes our failure to calculate coefficients of variation for tibia length (as we had done for sex-comb) and upon calculating such coefficients finds the greater variability of the inbred lots significant. But the same difference in variability was indicated by the standard deviations (which we gave) and the calculation of the coefficient of variation adds nothing to the force of the demonstration. We considered then and still consider the differences observed sufficiently accounted for by external conditions, *i. e.*, we considered them purely phenotypic. We showed that length of tibia is greatest and its variability least when food and temperature conditions are best. The difference between two inbred races (*M* and *N*) inbred practically the same number of generations (viz., 31 and 30, respectively) but treated very differently as regards food, was found to be several times greater than the difference between the inbred culture *M* and the not-inbred culture *X*. Hence it is not probable that the inbreeding had anything to do with the differences found in variability.

It is difficult to understand how on any theory of heredity inbreeding could be expected to *increase variability* within a single inbred line, such as one of our inbred cultures of *Drosophila*. On a Mendelian theory it would be expected that inbreeding, brother with sister, for a large number of generations (61 in our *A* series) would result in the production of a number of homozygous lines, each of which by itself would be *entirely devoid* of variability, except that due to environmental agencies. If all the possible derived lines descended from a pair under inbreeding were combined into one mass of material, it would seem probable, on a Mendelian theory, that if any genotypic varia-

tions had occurred, this material would show greater variability than the ancestral race before inbreeding began. This, I take it, is the point which Walton has in mind when he asserts that inbreeding has a tendency to increase variability. But this is very different from the condition to be expected in any *single line* considered separately, as in one of our inbred lines of *Drosophila.* Such a line should be *less variable* than the population from which it arose, provided that population contained any genotypic variations whatever!

The question is decidedly worthy of consideration, which Walton's paper suggests, is evolution more rapid in a self-fertilizing or habitually close fertilizing population on one hand or in a habitually cross fertilizing population on the other hand. The importance of the question is not lessened by the fact that Walton has brought into the discussion material wholly irrelevant, including his own observations on the zygospores of *Spirogyra* and the observations of Jennings on *Paramecium* and Barrows on *Drosophila.* But the work of Hayes on the variability of pure races of tobacco and of their hybrids, which Walton cites, does bear directly on this question. By combining the observations on the parent races into one mass of data and treating this statistically, Walton has shown that self-fertilizing lines *mixed together* would form a population more variable as regards number of leaves and height of plant than the population produced by cross-breeding of these same lines. Hayes's observations verify Focke's law already cited, that the variability of F_1 does not exceed that of the more variable parent race, but that F_2 shows increased variability. Theoretically F_2 should show the *maximum* variability. Walton's figures indicate clearly that this maximum variability under cross-breeding is *less* than the variability of a mixture of the two inbred races and consequently that continuous self fertilization within a mixed population will produce a more variable population than will result from continuous cross fertilization. This is an important generalization which demonstrably will hold good in all cases in which "intermediate" or "blending" inheritance occurs. It would not hold good for cases in which completely dominant and quantitatively invariable Mendelian factors are concerned, but it is doubtful whether such cases occur, as I have elsewhere tried to show. It is the great variability of self fertilizing populations and the stability of variations arising under self fertilization (since no variations will be "swamped by cross-

ing'' in a self fertilizing population) that allow of the formation and perpetuation of ''little species,'' side by side and yet quite distinct, within highly variable taxonomic species such as the dandelion. These same characteristics of self fertilizing populations furnish much of the material which plant breeders use. Following Vilmorin, they find it necessary only to *isolate* and propagate by themselves the variations which spontaneously arise. The task of the breeder who is dealing with a continually cross-breeding organism is much more complex. He often finds it necessary *first to inbreed* his stock, in order to learn what potential variations it contains, or, if one prefers so to express it, in order to induce variability, though this form of statement is not strictly accurate. Such inbreeding of a naturally cross-breeding organism often causes temporary loss of vigor, as notably in the case of maize, and frequently in domestic animals. But when the desired variations have been isolated, vigor can usually be recovered by increasing the stock to such an extent that matings become possible within the race and yet not involving union of closely related individuals.

Notwithstanding the utility of inbreeding in securing variations, there are important sources of variability which are found in cross-breeding alone. Supposing that under inbreeding variation has already occurred in different directions and the original condition has been wholly lost, it is often possible to recover it again by crossing. This is the familiar phenomenon of reversion upon crossing. It is also possible by crossing to combine in one race variations which have occurred separately in different races, a thing which would be impossible under continuous inbreeding. But a certain amount of inbreeding must usually in such cases follow up the cross-breeding in order to isolate and make secure the combinations desired.

It is not wise, therefore, unduly to exalt either inbreeding or cross-breeding as evolutionary processes or tools of the breeder. Each has its utility at the proper time and place. They are like pick and shovel, each supplementing the work of the other.

The question is worth considering in this connection—what effect will inbreeding and cross-breeding respectively have on the variability of single characters. This is a question to which I have given considerable attention for several years and the answer to it is, I think, becoming clear. A single character which Mendelizes has its variability increased by crossing. Some explain this as due to actual modification of the unit character

through crossing, others as due to the introduction of modifying factors by means of the cross. Whichever view is adopted, the fact is perfectly clear that modification of single Mendelizing characters occurs in cross-breeding. Under continuous inbreeding we should expect that single Mendelizing characters (within single lines but not within an entire inbred population) would attain a condition as devoid of variability as it is possible for them to attain and observation confirms this expectation.

As regards characters which "blend" in heredity, these are not inherited as single characters; they do not Mendelize in the ordinary acceptation of the term. The characters of the respective parent races *disappear* in the cross, being replaced by a common intermediate condition or blend. This blend persists into the F_2 and later generations but with a certain amount of variability which is at a maximum in F_2 and beyond that point tends to disappear in the absence of any special selection. It points to imperfection of the blending process or, in the view of those who prefer a Mendelian terminology for such cases, it points to *plurality* of factors determining the character. All the cases with which Walton has dealt in the paper under review are cases of *blending* inheritance and as regards them it is true, as already indicated, that continuous inbreeding tends to the production of a *more varied population* (but not of more variable separate lines) whereas cross-breeding tends to produce a *less variable population* (devoid of differences between families) but nevertheless a population more variable than the single lines of a self fertilizing or constantly inbred population.

W. E. Castle

Bussey Institution

NOTES AND LITERATURE

MIMICRY IN BUTTERFLIES

American biologists have been somewhat in a quandary of late as to what to believe and to teach about "mimicry" in insects. The consideration of chance resemblances in animate and inanimate things in which mimicry in the strict sense could not possibly exist, and the widespread skepticism of natural selection as an effective, creative agency in evolution have made many of us inclined to bury mimicry in the same grave with telegony, prenatal influences, the inheritance of acquired somatic characters, and sexual selection. Meanwhile, the Oxford school of zoologists, under Professor Poulton's leadership and the inspiration of an orthodox faith in the potency of natural selection, have continued to accumulate a rich array of newly discovered models and mimics among African butterflies.

Many of these and other cases of mimicry are described in the opening chapters of Professor R. C. Punnett's interesting book and admirably portrayed in the sixteen plates, twelve of which are in colors. With a remarkably clear and convincing style that has become familiar to us through his popular little book on Mendelism Punnett here recounts the history of the theories of Bates and Müller, mentions some of the morphological features upon which real affinities among butterflies depend, and describes in some detail examples of mimicry from various parts of the world.

Of particular interest to us in the United States is his brief discussion of the supposed mimicry of *Papilio philenor* by *P. troilus*, by the black southern variety, usually called *glaucus*, of the female of our common *turnus*,[2] and by a third species, *P. asterius* (usually known by us as *P. polyxenes*, or *P. asterias*). The northward extension of the range of *troilus* into Northwest Canada, far beyond that of the supposed model *philenor*, is thought to weaken this as a case of mimicry, and the author concludes that

[1] "Mimicry in Butterflies," by R. C. Punnett, Cambridge Univ. Press, 1915, 8vo., pp. 159, 16 plates.

[2] Punnett transposes these names, following Poulton (*vide Annals Entom. Soc. America*, Vol. 2, 1909, p. 225), who adopts Rothschild and Jordan's revision.

On the whole it seems at present doubtful whether any relation of a mimetic nature exists between *P. philenor* and these three species of *Papilio*.

The blue female of the southern fritillary, *Argynnis diana*, and our "red-spotted purple," *Limenitis* (*Basilarchia*) *astyanax*, which Professor Poulton has conceived also to be mimics of *P. philenor*, are likewise regarded as "very problematically mimetic." The striking resemblance of our "viceroy," *L.* (*B.*) *archippus*, for the "monarch," *Danais* (*Anosia*) *plexippus*, is mentioned, though no allusion is made to Abbott's biometrical study of 87 specimens of the supposed ancestral type, *L.* (*B.*) *arthemis*, from which the mimic, *archippus*, is thought to have arisen. Abbott,[*] by the way, found that the color markings involved in the Poulton hypothesis of gradual change by natural selection (*e. g.*, reddish spots) are much less variable than the blues and other colors not considered in that theory, the color pattern of *arthemis* showing no tendency to break up or to shift in the direction of the *Anosia* type.

Punnett next examines critically Wallace's well-known laws or conditions of mimicry, discusses the evolution of a Ceylonese "mimicry ring" (a group of five superficially similar butterflies), describes the case of *Papilio polytes*, the trimorphic "mimetic" and "non-mimetic" females of which are genetically separated from one another by two Mendelian factors, considers the enemies of butterflies, and, finally, the relation of seasonal and local variation to mimicry. He arrives at the general conclusion that there are two prominent difficulties in "accepting the mimicry theory as an explanation of the remarkable resemblances which are often found between butterflies belonging to distinct groups," viz., "the difficulty of finding the agent that shall exercise the appropriate powers of discrimination, and the difficulty of fitting in the theoretical process involving the incessant accumulations of minute variations with what is at present known of the facts of heredity."[3] In view of these difficulties, taking his cue from genetics, he suggests that

> Each group of Lepidoptera contains, spread out among its various members, a number of hereditary factors for the determination of color pattern. . . . Some factors may be common to two or more groups, in which case some of the permutations of the factors would be similar in the groups and would result in identical or nearly identical pattern.[4]

[*] Washington Univ. Studies, Pt. 1, No. 2, 1914.

[3] P. 139.

[4] Pp. 145, 146.

Thus, referring by way of illustration to the somewhat analogous case of the coat colors of rodents, he says:

In certain features the rabbit might be said to "mimic" the mouse, in other features the guinea-pig.

It is a significant fact in this connection that the various models "mimicked" by the different species of a polymorphic species are almost always closely related, and hence may be expected to exhibit color patterns based on different combinations of identical factors.

In criticism of Wallace's laws of mimicry, Punnett points out the fact that although the mimic and model usually occur in the same locality this is not always the case, the cooperation of migratory birds being invoked to explain the exceptions.

Regarding the defenselessness of mimics as compared with models, it is noted that the "mimic" is often a swifter flyer, and hence better prepared for defense than the model.

Exceptions are given to the rule that the models are more numerous than the mimics, and that the mimics differ from the most of their nearest allies. The Pierid genus *Dismorphia,* for example, includes prominent South American mimics which differ strikingly from the "whites" of the Temperate Zone but, unfortunately for the theory of mimicry, only about a dozen of the seventy-five described species are white, the rest presenting a "wonderful diversity of color and pattern." Among them are species clearly non-mimetic as regards color, which by simple substitution of one color for another in the spots would be transformed into a "mimetic" species.[5]

The author concludes that

It is on the whole unusual to find cases where a single species departs widely from the pattern scheme of the other members of the genus and at the same time resembles an unrelated species.

Two of the best examples are our American "viceroy" and the pierid *Pareronia.* "Mimicry tends," he adds, "to run in certain groups" and "in many cases at any rate little meaning can be attached to the statement that the imitators differ from the bulk of their allies."

[5] The reviewer recently observed in Porto Rico a case bearing upon this point, in *Leptalis* (*Dismorphia*) *spio*, which closely resembles in color and general shape the very common *Heliconius charitonius.* A color variety of the former, however, is found in certain localities on the island, in which orange replaces yellow in the color pattern, rendering the resemblance to the Heliconian less apparent. A simple mutation of orange into yellow would make this an excellent example of "mimicry."

In the chapter entitled "Mimicry Rings" the author considers the difficulty of explaining the protective value of the minute initial variations in the direction of a model. As an illustration, a group of five superficially similar butterflies in Ceylon is described. This "mimicry ring" includes two hypothetically distasteful Danaines (*D. chrysippus* and *D. plexippus*) and the females of three very unlike males (*Hypolimnas misippus, Elymnias undularis,* and *Argynnis hyperbius*). The coloration of one of these males (*E. undularis*) is a deep purple brown, like that of "satyrs" generally. If this represents the original type from which the gay orange and black pattern of the female has been derived, how has the change come about? Slight initial variations of the Satyr in the direction of the orange Danaine could not possibly be mistaken by birds for the model. The absurdity is pointed out of assuming, on the other hand, that the Danaine was originally like the male Satyr, and acquired its warning coloration *pari passu* with the mimic, for the Danaine model can hardly have been originally like all of the three very diversely colored males of the mimicking females. Mutation in each of the three types, however, may have produced females so similar to the Danaine as to be mistakable for it, and if natural selection indeed operates in this case, it may act in "putting on the finishing touches," or in preventing regression.

In the two following chapters the author discusses the resemblance of two of the three varieties of female *Papilio polytes* to the two "poison-eating" Papilios of India and Ceylon, *P. aristolochiæ* and *P. hector*. As is well-known, Punnett[6] has himself studied in Ceylon the behavior of these species, and Freyer[7] has continued the work, making extensive breeding experiments on the polymorphic "mimic."

A study of the geographical distribution in this case shows a general correspondence between the range of each mimic and its model, but notable differences are discovered.[8] Regarding the value of the resemblance between mimic and model, Punnett had no difficulty in distinguishing between model and mimic on the wing, even at a distance of forty to fifty yards, while near at hand the brilliant scarlet of both models, which covers the body and is conspicuous in spots upon the wings, is seen to be very different from the softer red found upon the wings (not

[6] "Spolia Zeylanica," Vol. 7, Part 25, 1910.

[7] *Phil. Trans. Roy. Soc.*, London, Vol. 204, 1913.

[8] *Vide*: Lutz, American Naturalist, Vol. 45, p. 190, 1911.

upon the bodies) of the mimics. Dried specimens of models and mimics are likely to be confused, but not the living butterflies.

Freyer's breeding experiments bring out the fact that a simple Mendelian relation exists between the three varieties of female in *P. polytes*, the males of which, though phenotypically alike, correspond genotypically to the three kinds of female. Of these three the one resembling the male ("non-mimetic") is recessive to the mimetic forms, lacking a factor, X, possessed either in simplex or duplex condition by the "mimetic" females. The male likewise is latently either xx, XX, or Xx, as the case may be, but retains a uniform appearance in all cases owing to the presence of an inhibitor factor for which he is heterozygous (Ii), the female being recessive (ii). The male, unlike other Lepidoptera, so far as they have been investigated, is also suppos͏d to be heterozygous for a sex factor which we may for brevity call M, responsible for maleness, with which the inhibitor factor is completely coupled, so that the male-producing sperms (MI) always contain the inhibitor factor, the female-producing always lack it (mi).

The two mimetic varieties of female are differentiated from each other by the presence or absence of another factor, Y, which acts merely as a modifier of the factor X when that is present, and transforms the aristolochiæ-like female (XXyy or Xxyy) into the hector-like (XXYY, XXYy, XxYY, or XxYy). This color modifier, responsible for intensifying and extending the red markings, is supposed to occur in either homozygous or heterozygous condition, or to be absent (recessive) in the male-like form of female and also in each biotype of the male, though when present without X, or in the presence of I, it has no visible effect. Thus there are 9 biotypes of males and 3 of male-like females, all phenotypically alike. Referring to Poulton's theory of the gradual evolution by natural selection of the male-like type of female into the aristolochiæ-like, and subsequently into the hector-like, Punnett argues that crossing the hector-like (double dominant) with the male-like (double recessive) germ plasms and inbreeding should show the hypothetical intermediates postulated by Poulton, but no such intermediates have appeared in the breeding experiments.

Freyer's random sampling of the population of *polytes* gave 49 of the two mimetic females to 40 of the male-like coloration, or roughly 5 dominants to 4 recessives, a proportion indicating

stable equilibrium between the mimetic and non-mimetic varieties. Scanty historical data tend to show that the mimics were as common fifty years ago, and probably a century and a half, as to-day, so the author concludes "that in respect of mimetic resemblances natural selection does not exist for *P. polytes* in Ceylon," or at least there is "no effect appreciable to the necessarily rough method of estimation employed."

The author next considers the evidence that the enemies of butterflies could have played the part assigned to them by the advocates of the mimicry theory. Predaceous insects evidently pay no attention to warning colors; certain lizards devour butterflies freely, but do not exercise any discrimination in the species which they attack. Hence neither insects nor lizards can be supposed to play any part in establishing a mimetic resemblance. Birds destroy butterflies in considerable numbers, but

> Some of the most destructive appear to exercise no choice in the species of butterfly attacked. They simply take what comes first and is easiest to catch.

Monkeys and baboons often eat butterflies. They show strong likes or dislikes for certain species. The monkey may be regarded as "the ideal enemy for which advocates of the mimicry theory have been searching—if only it could fly." The conclusion is reached that

> even a slight persecution directed with adequate discrimination will in time bring about a marked result where the mimetic likeness is already in existence. It is not impossible therefore that the establishing of such a likeness may often be due more to the discrimination of the monkey than to the mobility of the bird.

In the final chapter on "Mimicry and Variation" the author describes Carpenter's observations on the polymorphic mimic *Pseudacræa eurytus,* the four forms of which show an extraordinary resemblance to acræine "models" of the genus *Planema.* These butterflies inhabit the shores of Victoria Nyanza in Central Africa where the models are very abundant, the polymorphic mimics less common but still numerous, and intermediates between the different types of mimic rare, but not unknown. On Bugalla Island in the lake, on the contrary, the mimetic Pseudacræas are very abundant, and their respective Planema models relatively rare. Here intermediates between the varieties of the polymorphic mimic occur in proportionately larger numbers than on the mainland, owing as Dr. Carpenter believes to the

cessation of natural selection in the absence of sufficient models to familiarize the hypothetical enemy with the several warning color patterns of the models. On the mainland, however, any of the aberrant intermediates that might be produced by interbreeding of the different varieties of the polymorphic species would meet an enemy having constant experience with the warning colors of the different models, and tend to be eliminated. The enemy, in other words, would avoid the perfect mimics, while aberrant individuals suggesting two models at once presumably would be attacked and eaten. This interesting case deserves thorough investigation.

The author makes a *faux pas* when he says regarding seasonal variations in butterflies, due to "changes in the conditions of later larval and earlier pupal life":

> In no case are they known to be inherited, and in no case consequently could variation of this nature play any part in evolutionary change.

In the example cited (*Araschnia levana-prorsa*), which presents two color patterns alternately through the year, it is obvious that both patterns are inherited. The environment indeed decides which shall appear, but the hereditary basis common to both seasonal types is no less real than that of any butterfly of seasonally uniform pattern. Although the seasonal color patterns of *A. levana* and *prorsa* apparently can not behave as Mendelian allelomorphs to one another as do the color patterns of other non-seasonal polymorphic insects, they are by no means outside the pale of Mendelian heredity. It is not too much to expect that future studies will disclose colors or color patterns allelomorphic to *A. levana-prorsa's* shifting coloration. The reviewer would not, with Professor Punnett, rule seasonal variation entirely out of court as possible stages in "the development of a mimetic likeness" or, rather, in the evolution of the remarkable likenesses, alleged to be mimetic, which this book brings so well to our attention.

The author is so strongly influenced by the idea that minute variations are fluctuations always controlled by the environment rather than by the internal conditions that result in heredity that he treads upon uncertain ground in discussing an example of local variation cited by Poulton.[9] A small white spot on the wing of *Danais chrysippus* varies in size locally from a conspicuous marking, in China, to a faint dot tending to dis-

[9] Bedrock, 1913, p. 300. Cited by Punnett.

appear, in Africa. Punnett suggests that the details in pattern may be in slight measure affected by the plant species on which the caterpillars have fed, thereby producing local races. Transportation of a local race to a region inhabited by another distinct local race "would help us in deciding whether any variation by which it is characterized had a definite hereditary basis or was merely a fluctuation dependent upon something in the conditions under which it had grown up." We may well ask: are these two propositions mutually exclusive? May not a detail of color pattern to a certain degree at certain times be subject to environmental influences and at the same time may not its variations have a "definite hereditary basis?" The reviewer has had so much experience in observing the transmission in *Colias philodice* and *C. eurytheme* of spots comparable to that mentioned in *D. chrysippus* that he is convinced that a definite hereditary basis (consisting presumably of multiple factors) underlies every fluctuating detail of color pattern. By artificial selection from inbred stock, using uniform food plants, and exposing the caterpillars and pupæ to similar conditions, the breeder of butterflies may decrease even to elimination or increase within certain limits a detail of color pattern like that mentioned. The champions of the theory of mimicry are entitled to this crumb of comfort. "For if it can be supposed," remarks the author, "that small differences of this nature are always transmitted, it becomes less difficult to imagine that a mimetic resemblance has been brought about by a long series of very small steps."

Yet the facts which the mimicry theory seeks to explain clamor for explanation. Punnett sets forth at the end some that are most insistent.

> Certain color schemes are characteristic of distinct geographical regions in South America, where they may occur in species belonging to very different genera and families.

In Central America a pattern occurs that is common to several Heliconines, Ithomiines, Nymphalines of two or more genera, and Pierids; in eastern Brazil another pattern in which "all the various genera which figure in the last group are again represented." On the upper Amazon a still different pattern is common to the same group of genera from that just mentioned, only two notable genera being absent. Finally in Ecuador, Peru and Bolivia a widely different pattern occurs in a group lacking

a Pierid or Danaid but containing in exchange "a *Papilio*, an *Acræa*, and two species of the Satyrid genus *Pedalodes*."

Assuming that one of these patterns must have been the most primitive, he asks why a distasteful genus should change from one efficient warning pattern to another quite distinct one. Though the premise is not necessarily true nor even probable, yet if the ancestral pattern were a generalized type of any "warning" character whatever, the question would still be pertinent.

The author suggests that a newly acquired color scheme, like one of these, may be "associated with a certain physiological constitution which places butterflies possessing it at an advantage as compared with the rest," just as the melanic variety of peppered moth that is ousting the typical form in Britain and on the Continent may have associated with its deep pigmentation a greater hardiness. This, however, goes but a short way toward the explanation of the extraordinary local associations of unlike South American butterflies showing similar coloration. This is a live question that challenges the attention of any student of evolution who has opportunity to undertake experimental work in the tropics of South America.

So if we must sooner or later consign Mimicry to its last resting place, with its less infirm but already moribund parent, Warning Coloration, let us do so filled with gratitude for the pioneer work accomplished by its champions in opening up promising fields of investigation, where we or our descendants may hope to discover new factors in evolution or to gain a deeper insight into those now only dimly understood. Thanks are meanwhile due to the author of this attractive volume for his keen diagnosis of the present condition of the mimicry theory and for his admirable description of the phenomena which it has attempted to explain.

John H. Gerould

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Bound in Buckram. Royal 8vo. Pp. viii+188. With 16 plates (12 of which are colored). Price $4.50.

By Reginald Crundall Punnett, F.R.S., Fellow of Gonville and Caius College, Arthur Balfour Professor of Genetics in the University of Cambridge.

EXTRACT FROM THE PREFACE

"This little book has been written in the hope that it may appeal to several classes of readers.

Not infrequently I have been asked by friends of different callings in life to recommend them some book on mimicry which shall be reasonably short, well illustrated without being very costly, and not too hard to understand. I have always been obliged to tell them that I know of nothing in our language answering to this description, and it is largely as an attempt to remedy this deficiency that the present little volume has been written.

I hope also that it will be found of interest to those who live in or visit tropical lands, and are attracted by the beauty of the butterfly life around them."

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THE
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THE MECHANISM OF CROSSING-OVER

HERMANN J. MULLER

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It is the object of this paper to give an account of the most important evidence thus far gained in regard to the manner in which separation of linked factors—often interpreted as "crossing-over"—takes place, and to describe an experiment in which a new method for studying the occurrence of such separation is employed. This experiment is still under way, but as it may be a considerable time before the results are obtained in full, it would not be advisable to withhold longer an account of this work and of other work that bears on the nature of the "crossing-over."

I. The Discovery of Interchange between Homologous Chromosomes

The question as to whether separation of linked factors is due to pieces of homologous chromosomes changing places with each other, carries us back to the question whether the factors lie in the chromosomes at all. As is well known, there is a large body of evidence from cytology and from experimental embryology, showing that the chomosomes are persistent, self-perpetuating structures which have a profound influence upon development. But the first definite evidence that the Mendelian factors are contained in the chromosomes lay in the striking correspondence which was found between their respective

methods of distribution—the segregation of Mendelian allelomorphs exactly paralleling the pairing and separation of homologous chromosomes during the maturation of the germ cells, and the random assortment of Mendelian factors belonging to different, independently segregating pairs, paralleling the random assortment of chromosomes belonging to different pairs (Sutton). Still, there was no indication of a connection between any particular chromosome and a particular character until the work of McClung, Stevens and Wilson and others proved that in many animals the "X-chromosome" contains, or at least invariably accompanies, a factor for sex, inasmuch as all fertilized eggs which receive two X-chromosomes develop into females, while those with one X become males.

The next time that particular factors and chromosomes were found to be correlated was in 1910, when Morgan pointed out that the factor in *Drosophila* determining whether an individual shall have red or white eyes, as well as several other factors, must also be located in the X-chromosome (or at least must *accompany* it in its segregation). For, to put the whole argument briefly, the fact that a red-eyed male bred to a white-eyed female produces red-eyed daughters and white-eyed sons shows that the female-producing spermatozoa—those that receive the X-chromosome—also receive the factor for red, but the male-producing sperm—which do not receive the X— also fail to receive red. In other words, the factor for red was judged to be in the X-chromosome, because in the male it is always distributed to precisely the same spermatozoa as those to which the X's happen to be distributed. Bridges has recently obtained evidence that in the female, too, such "sex-linked" factors accompany the X-chromosome in segregation. Ordinarily there is no opportunity for attacking this question in the female, since the female contains two X's, which are of course indistinguishable to the eye, so that it would be impossible to tell whether or not a particular one of the X's was always distributed to the

same eggs as a particular sex-linked factor. But in Bridges's cases of non-disjunction, the maturation divisions are often abnormal, so that some eggs are found to have retained both X's; and in accordance with this it is found that some of the offspring of such females have likewise received two sets of maternal sex-linked factors. These cases, therefore, show that in the female also the sex-linked factors "follow" the X-chromosome (1, 2).

Morgan next studied the relation of different sex-linked factors to each other in inheritance, and then another remarkable fact came to light. Theoretically, the dihybrid females resulting from a cross of a red-eyed fly having rudimentary wings by a white-eyed fly having long wings (both of these pairs of characters are sex-linked) should have contained in one of their two X-chromosomes the factors "red" and "rudimentary," and in the homologous X-chromosome the factors "white" and "long"; the mature eggs should retain either one X or the other and should therefore have contained either red and rudimentary or white and long. In other words, red and rudimentary should be *completely linked* in their inheritance, and similarly white and long. But the results showed that these factors sometimes separate in heredity, for not only the above types of offspring are produced, but also some red longs and white rudimentaries (7); in fact, about 42 per cent. of the offspring belong to one or the other of the two latter classes. If we admit that white and long were originally present in the same chromosome, the *only* way to account for this separation of the factors is to suppose that in some of the cells of the hybrid female the X-chromosomes interchanged parts before being distributed to the eggs. For if the factor for "long" of the chromosome containing "white" and "long" should somehow change places with the "rudimentary" of the homologous chromosome, then when homologous chromosomes are separated at the maturation division, the egg may come to contain either an X-chromosome with white and rudimentary or an X with red and long.

Recent work on *Drosophila* has borne out in a striking way the conclusion that the separation of factors just discussed is due to chromosomal interchange. It will be remembered that the pairs of factors in the example underwent recombination in only about 42 per cent. of the eggs, *i. e.*, they held together more often than they separated, and so might be said to be *partially linked*. Their mode of inheritance therefore forms a contrast not only to complete linkage, but also to the familiar cases of random assortment, where two pairs of factors are found recombined in about 50 per cent. of the offspring, and thus show no linkage at all, presumably because they lie in different pairs of chromosomes which segregate independently. Further investigation showed that not only "white" and "rudimentary," but all the known sex-linked factors, instead of segregating independently, are "partially linked" to one another in greater or less degree. This then was additional evidence that these factors did not lie in different pairs of chromosomes, as in familiar cases, but in the same pair of chromosomes, and that their separation or recombination was therefore dependent upon chromosomal interchange. But furthermore, if these linked factors all lie in the X-chromosome (being sex-linked), then it might be expected that other groups of interlinked factors also would be found, that lie in other chromosomes. A factor in any one of these other groups would not be sex-linked, but would be linked in greater or less degree to every other factor of the same group, since it lay in the same chromosome with it, but it would undergo 50 per cent. of recombination with factors in other groups. This expectation has been fulfilled. In 1911 Morgan and Lynch found two pairs of factors in *Drosophila* (black versus gray body color; vestigial *vs.* long wings) that were linked to each other, but that were not sex-linked (10). These were designated as lying in group II or Chromosome II. Later, Sturtevant found that two other pairs of factors (pink *vs.* red eyes and ebony *vs.* gray body color) were also linked to each other, but were neither sex-linked nor linked to the

other non-sex-linked group; these were assigned to Group III or Chromosome III (15). Incidentally, it was evident that these cases are of exactly the same nature as those previously discovered by Bateson and Punnett in the sweet pea, and termed by them "coupling" or "repulsion." Moreover, the chromosome interpretation made it clear why the factors should be "coupled" or "repelled" according to whether the hybrid received them from the same or from opposite parents. There was only one difference in detail between the facts in the two species: it was discovered by Morgan that in *Drosophila* the linkage is always complete in the male, the separation of factors that are linked to each other occurring only in the female (9); in the plants, on the other hand, recombination occurs in the genesis both of eggs and of sperm.

Since that time the inheritance of over one hundred pairs of factors of *Drosophila* has been studied. This investigation should give an extensive experimental test of the theory of chromosomal interchange, for if linked factors are those carried by the same chromosome, there should be the same number of groups of interlinked factors as there are pairs of chromosomes. There are *four* pairs of chromosomes in *Drosophila*—two pairs of long ones, the pair of moderately long sex-chromosomes, and a pair of very small chromosomes. By 1913, work had been done upon a large number of factors, and the results showed *that all these factors were linked in one of the three groups already discovered.* But in 1914 the author found a pair of factors independent of these (bent *vs.* straight wing), *i. e.,* constituting Group IV (12), and not long afterwards Miss Hoge found another pair of factors in this fourth group (eyeless *vs.* normal eye), (3). Accordingly, the number of groups and of chromosomes now correspond, and not only that, but the relative sizes of the groups correspond in a general way with the relative lengths of the chromosomes. Can it be mere chance that one hundred factors fall into this particular grouping? But if it is admitted that these groups are carried in the

chromosomes, then, as above pointed out, the separation of factors in a group means chromosomal interchange.

II. A Mechanism of Interchange already provided by the Theory of Crossing-Over (Chiasmatype)

Janssen's "chiasmatype theory," based on cytological observations of spermatogenesis in *Batracoseps*, described just such a process of interchange between the homologous chromosomes as Morgan's evidence from genetics required (4). A great bulk of evidence has accumulated to show that during the period of synapsis, homologous chromosomes come into contact, and in many cases chromosomes can be seen to be twisted around each other during one stage or another of synapsis. The essential point postulated by the chiasmatype theory is that, as the paired chromosomes draw apart again, they do not always untwist completely, but may break at some points where they are crossed—thus, in Fig. 1, the upper piece of the light-colored chro-

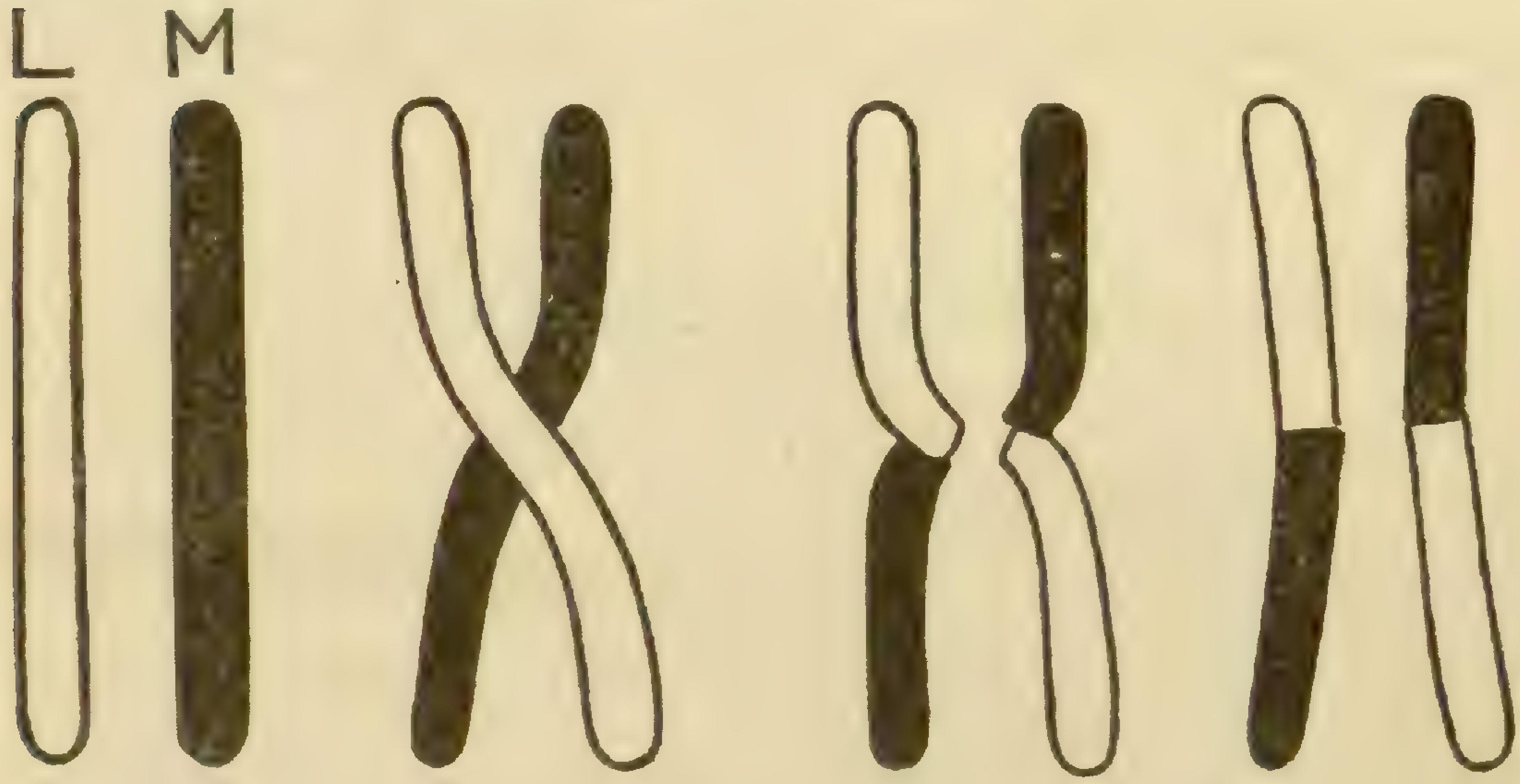

Fig. 1.

mosome (L), which was on one side, loses its connection with the lower part of L, that has crossed to the other side, but becomes united instead with the lower piece of the dark chromosome (M) which, on account of the crossing of the strands, now follows it on the same side; similarly, of course, the upper part of M becomes united with the

lower part of L; in this way a recombination of parts is accomplished. Morgan and other workers on *Drosophila* base their acceptance of this essential point in Janssens's chiasmatype theory upon the evidence (from cytology) that homologous chromosomes do twist about each other during synapsis, taken together with the evidence (from genetics) that these chromosomes emerge as new combinations. Janssens, on the other hand, maintains that certain details in the appearance of the chromosomes during that stage in synapsis called "strepsinema" give ocular evidence that crossing-over occurs at this particular period and in a particular manner. As it would seem possible, however, to put another interpretation upon his figures, this question may be deferred until later.

Janssens had intended the chiasmatype theory to explain the supposed fact that there might be more pairs of factors

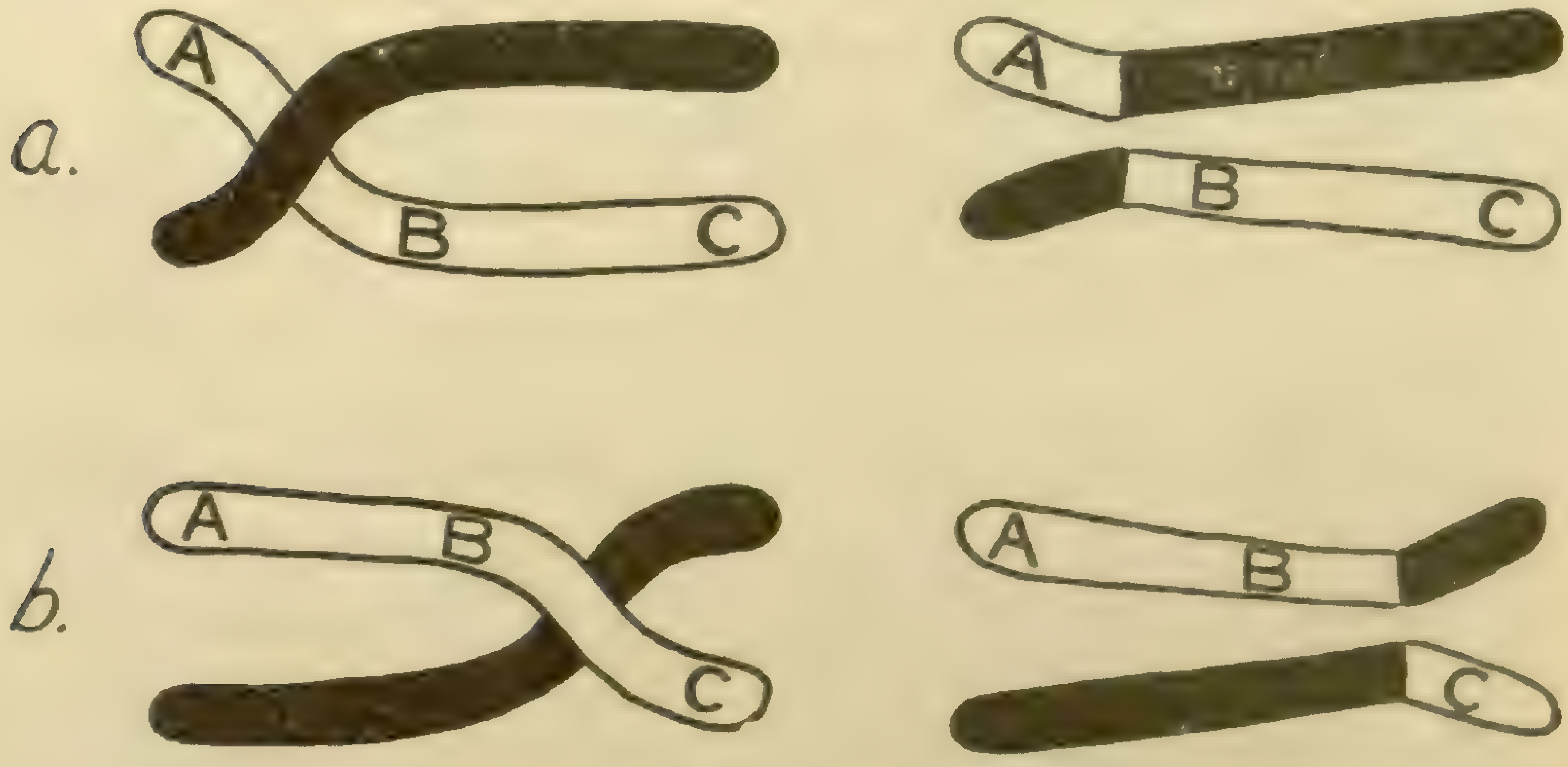

Fig. 2.

capable of recombination than there were pairs of chromosomes. (It might be mentioned in passing, however, that at that time this fact had not yet been demonstrated; there are even now probably no published facts except those recently discovered in *Drosophila* which prove this point). As shown above, Morgan went further than this with the chiasmatype theory by applying it to explain, specifically, the recombination of *linked* factors (8). Moreover, he pointed out at the same time an important corol-

lary to this theory. It has already been stated that he had found different degrees of linkage to exist between the various factors of a group: for example, the proportion of cases in which separation occurs between white and rudimentary was said to be 42 per cent., whereas the frequency of separation between white (eye color) and the factor for yellow body color is only about 1 per cent. In explanation of these different degrees of linkage, Morgan pointed out that, on the chiasmatype theory, the closer the proximity of two factors to each other in the chromosome, the smaller would be their frequency of separation, for the less would be the chance for a crossing-over of the chromosomes to occur *between* them. Thus, in Fig. 2, the factors A and C become separated both in case (*a*) and in case (*b*), because A and C lie so far apart that in both cases the point of crossing-over falls between them, but in only one of the cases do A and B separate, and in one case B and C, since these are so near together that the point of crossing-over may often be beyond instead of between them. In other words, on the chiasmatype theory, frequency of recombination must be, to a certain extent at least, an index of the distance apart of factors along the chromosome. Since the time when these ideas were proposed (1911), two important series of facts have come to light in the studies on *Drosophila,* in support of the chiasmatype theory of interchange and of these extensions of it.

III. A Verification of the Theory of Crossing-Over. The Law of Linear Linkage

It occurred to Sturtevant in 1911 that, if the factors are carried in the chromosomes, then, *owing to their linear arrangement*, the distance along the chromosome between any two factors (A and C) must be either the sum or the

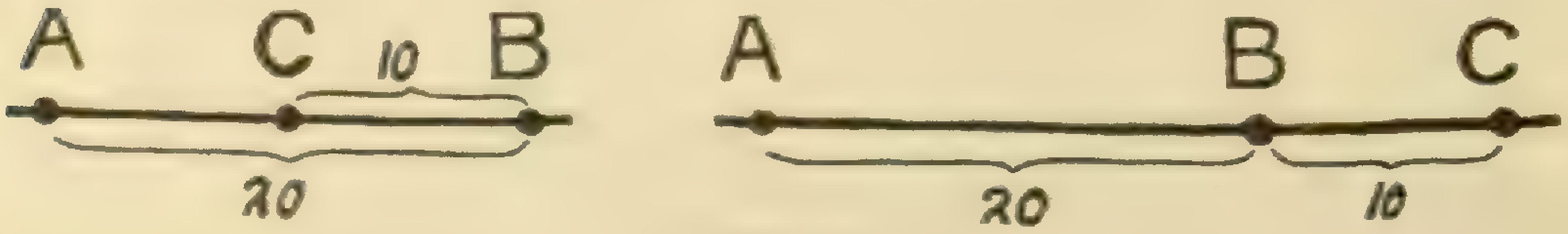

FIG. 3.

difference of their distances from any third factor (B) of the same group, *i. e.*, length AC = length AB ± length BC, the + or − depending on whether the third factor is between or beyond the other two (see Fig. 3). Accordingly, if, as Morgan suggested, the frequencies of separation (linkage values) between factors depend on their distances apart, then the frequency of separation (degree of linkage) between the two factors, A and C, should be predictable, given the frequency of separation of each from the third factor, B. To put the matter diagrammatically, A, B and C have been represented in figure 3 as points along a line; A and B, we will suppose, separate from each other in heredity in 20 per cent. of cases, to correspond with which they have been placed the same number of units (20) apart in the diagram; similarly, B and C, which we will suppose to separate 10 per cent. of the time, have been placed 10 units apart. (As above pointed out, there are obviously two possible diagrams to choose between, depending on whether C is beyond A and B or between them.) Then, if it be true that the frequency of separation between any factors is always precisely proportional to their distance apart, it will follow that the per cent. of separations between A and C will be equal to the number of units of distance on our diagrammatic chromosome between A and C; this in turn equals AB ± BC = 30 or 10. If separation frequency bears a less simple relation to distance, but is nevertheless determined by it (see below), frequency AC will not *equal* distance AC (*i. e.*, AB ± BC) but can be *calculated* from the latter. On the other hand, if our premises are false, and there is no linear relation at all between the factors that determine their frequency of separation, then frequency AC will not be equal to diagram distance AC (*i. e.*, to AB ± BC), nor even, in the case of different sets of factors, will it bear any constant relation to diagram distance AC; that is, it would not be possible to discover any constant rules for calculating the third frequency from the two others which will hold, even approximately, for various sets of factors (BCD, LMN, etc.).

Sturtevant found that there is indeed a linear relation in the frequencies of separation (14, 16). In the case of smaller per cents. of separation, per cent. AC always is precisely equal to the sum or difference of per cents. AB and BC (within the limits of probable error), so that the per cents. of separation for all combinations of these factors is accurately represented by a linear diagram. In the case of higher per cents. of separation (long distances), the highest of the three frequencies (let us call it AC) falls short of the sum of the other two (AB + BC), and so it is a smaller number than the distance representing it on the diagram, but it nevertheless (within the normal limits of error) can be calculated from this diagram distance AC, for a constant relation was discoverable between this hypothetical distance and the actual frequency. Thus the different frequencies do not bear any random relation to each other that is mathematically possible, but bear relations that disclose a linear connection between the factors.

It remains to consider the meaning of the fact that in cases where there is a high per cent. of separations, the highest per cent.—that between A and C, let us say—is not as great as the value of the distance AC representing it on the diagram, *i. e.*, it is less than the sum of the per

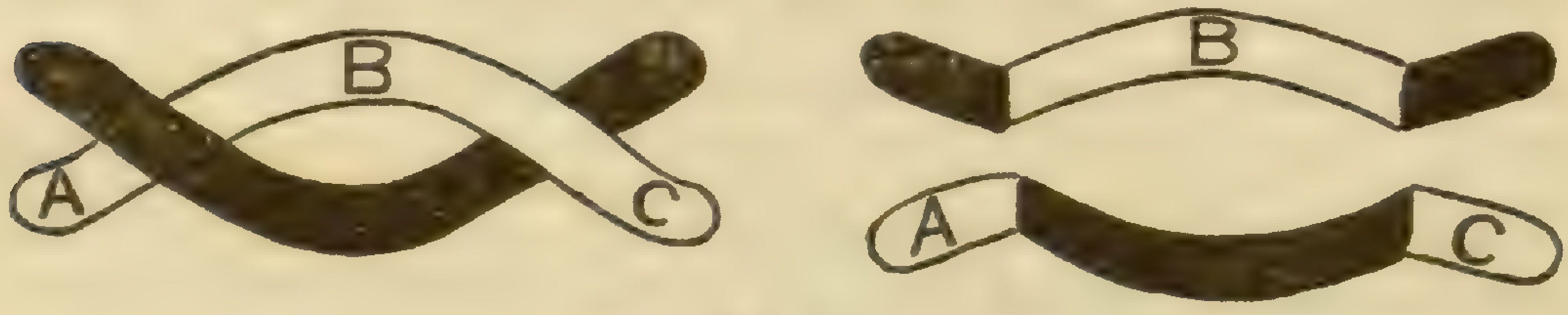

FIG. 4.

cents shown between A and B, and B and C, respectively. If, whenever A and B or B and C separate, A and C separate also, as shown in Fig. 2, (*a*) and (*b*), then per cent. AC would be equal to per cent. AB plus per cent. BC, but since per cent. AC is lower than this, there must be cases in which, although A and B or B and C separate, A and C fail to separate. It is obvious that in these cases, where B separates from A but A does not separate from C, that B

must have separated from C also, *i. e.*, a separation has occurred between A and B, and between B and C, coincidentally. On the view above presented, a separation means a crossing-over of chromosomes, and so in these cases the chromosomes must be thought of as crossing-over at two points coincidentally, as shown in Fig. 4. This process has been named by Sturtevant "double crossing-over." As shown in the figure, where crossing-over occurs coincidentally, both in AB and in BC, the chromosome crosses and crosses back again between A and C, hence the latter factors do not become separated.

When the frequencies of separation (diagram distances) between A and B, and between B and C are both small, it is to be expected as a matter of pure chance, if the factors are joined in line in the manner described, that such coincidences will occur very rarely, even in proportion to the small frequencies involved, and so the per cent. of separations between A and C will be practically as great as that between A and B plus that between B and C. Hence per cent. AC will be accurately represented by diagram distance AC. On the other hand, if separation is frequent between A and B or between B and C, there should be more chance of coincidence of these separations, and the number of separations between A and C will fall correspondingly short of AB + BC, which is the value of AC shown on the diagram. Consequently, in predicting frequency AC on the basis of AB and BC, allowance must be made for these coincidences. But the author ventures to point out that, as the number of these coincident separations is found to be largely determined by the frequency of separation, greater frequencies being accompanied by a larger proportion of coincidences, as has been shown, then the amount of allowance to be made can be approximately calculated for any given distance on the diagram; accordingly, the frequency of separation between A and C can be calculated from AB + BC, *i. e.*, from the distance AC on the diagram. The precise manner in which coincidence of separations increases with their frequency is a

question which will be reserved until later. But it is clear that, since coincidence does not vary independently of "distance," a linear relation holds between the linkage values, in that these values can be calculated from a linear diagram much more exactly than would be expected on chance relationships; in mathematical terms, the frequencies of separation between all combinations of the different factors in a group are largely a *function* of the distance apart of these factors in a linear figure.

It will now be desirable to consider these same facts from another angle. As it is possible to represent the linkages between *any three* factors of a group in terms of their distances on a linear diagram, it follows that *all* the factors of a group together can be represented in one linear diagram. Suppose that such a diagram has been made, and that the order of the factors in it is ABCDEFG. Now, as has just been explained, since per cent. AC nearly equals per cents. AB plus BC, it must follow that a separation between A and B rarely coincides with one between B and C; the same fact may also be expressed by saying that when A and B separate, C stays with B rather than with A. Similar relations, of course, hold for the other factors, too; thus D also stays with B and C when A and B separate, but it stays with C when B and C separate. The linkage of D with B, then, is only due to its linkage with C, for, although it usually stays with B, it very rarely stays with it except when C does. Thus D is linked to B only through C, and to A only through B. Similarly, all the other factors also are linked together in a chain, each to the one on either side: just as D is linked on the one hand to E, and on the other hand to C, but not to any other factors except through one of these, so C is linked on the one hand to D, on the other hand to B, but is linked to E only through D, and to A only through B, etc.; moreover, all the factors are linked to the others in the same order. Separation of factors in such a group accordingly means the breaking of the chain at just one or two points, for it has been pointed out that when B and C separate, A and

B rarely separate coincidentally, but usually remain together, and C, D, E, etc., all remain together also, separating in a body from A and B. In other words, the factors are not interchanged singly, but stay together in sections, according to their positions on the diagram, and whole sections are exchanged at once.

It may be objected that these conclusions are in many cases based on linkage values obtained in different experiments; that it is unwarranted to conclude, for instance, that when C and D separate, E remains with D, simply from the fact that in one experiment the frequency of separation between C and D had the value m per cent., in a second experiment DE was n, and in a third experiment CE was m + n, for this conclusion would only be true on the supposition that in all three experiments each factor had the same frequency of separation with each of the others as in the particular experiment where that frequency was determined. The answer is that numerous experiments have been performed in which three pairs of factors (or more) could be followed at the same time, and these experiments have given results precisely the same in kind, although more accurate than the preceding. But in experiments of the latter type, the coincidence of the various separations and non-separations does not have to be calculated out as in the case above, but is given directly by the results. Thus in a hybrid which has received ABC from one parent and the allelomorphs abc from the other, gametes in which coincident separation between B and A and between B and C has occurred will be distinguishable by having either the composition aBc or the composition AbC (see Fig. 3), and the number of such offspring can thus be directly counted instead of it being necessary to calculate them from the relations between separation values for A and B, B and C, and A and C. And in the experiment of the author's given in Section V, where the inheritance of a large number of factors is followed simultaneously, the results show directly and graphically that

the factors, as arranged in line in order of their linkage, are exchanged in whole sections at a time.

In the first section, evidence was presented, showing that groups of factors are connected with particular chromosomes, and segregate with them at the maturation divisions; this was in fact *proved* to be true in the case of sex-linked factors, which are found always to segregate with the X-chromosome during spermatocyte divisions. Yet it was conceivable that the factors were not actually *in* the chromosomes, but rather tied to them by some obscure connection (chemical, physical or metaphysical), although the fact that the relative sizes of the groups correspond to the lengths of the chromosomes might be taken as evidence against such a view. On that view, a separation of linked factors would be considered not a physical interchange between the chromosomes themselves, but a transference, by a factor, of its invisible bond, from one chromosome to the homologous one. But Sturtevant's evidence just presented shows that however one may have conceived, *a priori,* the chemical attraction or physical connection that makes linked factors tend to segregate to the same pole in the maturation divisions—this connection binds them in a *linear manner,* one after another, in a chain. This unique result, then, constitutes specific evidence that the factors are actually in the chromosomes, in an order which can be determined by their linkage relations, and that the separation of linked factors is consequently a real interchange between parts of the chromosomes themselves.[1]

[1] The fact of linear linkage does not connote that frequency of crossing-over is necessarily entirely dependent upon distance, for it is still possible to escape the conclusion that crossing-over occurs equally often in all parts of the chromosome, by assuming that coincidence of separations A–B and B–C usually occurs with not very different frequency from coincidence of separations G–H and H–I, even if different actual lengths are involved, provided the frequencies of separation are the same in the two cases. And on either way of explaining the results, the factors must be linked in line in the chromosomes in the same order as on the diagram. In fact, no matter how great the differences in frequency of crossing-over in different parts of the chromosome might be, the linkage order of the factors would still be the same as their real order so long as coincident crossing-over in any two regions did not occur as often as single crossing-over in either region.

Furthermore, admitting this conclusion that the linkage diagrams really represent the chromosomes, the fact that the factors are exchanged in sections proves that whole pieces of the chromosomes change places at once, as occurs in the process of "crossing-over" postulated by Jannsens, instead of small parts or factors in the chromosomes being separately exchanged. The idea that the interchange during synapsis may be a kind of exchange of separate particles from one container to another seems to have been held by a number of geneticists. On this view, the chromosomes might be considered as a sort of pod, containing the factors within them like so many beans; when the chromosomes synapse, the pods open towards each other, so that a factor in one might change places with a factor in the other. Conceivably—if we adopt this view—certain factors might be harder to dislodge than others, and so different frequencies of separation would exist between different factors. But such a mechanism of interchange would not result in a mode of linkage that may, in the sense explained above, be called linear, for separation of factor B from A would, on this mechanism, have no influence at all on whether or not C separated from A. This difficulty could be partially met by supposing that interchange of one factor in some way facilitates interchange of the neighboring factors, but the type of linkage which is actually found goes much further than this, and shows that the whole group of factors remains intact except at one or two points, interchange being in two or three entire sections. This can only mean, then, that interchange is a process of *crossing-over,* if it occurs by means of synapsis at all.

It might be claimed, however, that this interchange of whole sections of the chromosomes need not occur at synapsis, and therefore need not be of the nature of crossing-over at all. The only alternative to crossing-over, however, would be to suppose that, during the resting period of cells, the chromosomes might break up into pieces, and that then, in reuniting, a fragment of one chromosome

might become joined with a piece of the homologous chromosome instead of with a piece of the same chromosome. But on the fragmentation theory it must be supposed that the fragments reunite in exactly the original order, and, further, that the two homologous chromosomes break at precisely the same point before interchanging—otherwise one reformed chromosome would lack certain factors and the other would have too many; nevertheless, this point can not be a fixed point, as interchange may occur anywhere. Since interchange, when it occurs, usually takes place at one point only, it must also be assumed that the frequency of the recombination just described is so nicely regulated that in about half of the cases it has happened just once (and at one point in the chromosome) during the sum total of resting periods of all cells ancestral to any particular egg cell that shows interchange. For in about half the eggs a particular chromosome has exchanged in only two sections, and in very few have there been more than three points of interchange. Moreover, in the ancestry of the rest of the eggs, no interchange whatever can have occurred. Finally, the fallacy of the fragmentation idea becomes obvious when we consider that if interchange took place in the resting period of an embryonic cell, most of the eggs derived from this cell would show that particular recombination, and hence the individual in question would give an unusually large proportion of offspring of this sort. Thus different individuals of the same strain would differ greatly in their linkage values, there being scarcely any constancy at all. Since this is not true it would have to be assumed that interchange takes place only a short time before the maturation divisions, owing to some peculiarity in the chromosome processes occurring in the cells at this period. Thus we return again to the conclusion that interchange occurs during synapsis.

Further evidence that interchange occurs during synapsis is to be found in some results obtained with Bridges' "non-disjunctional" flies. Non-disjunctional females of

Drosophila contain, besides their two X-chromosomes, a Y-chromosome (owing to previous mitotic abnormalities). The presence of the extra homologous chromosome in some way causes the X's, in some of the oocytes, not to separate properly at the reduction division (presumably, this is because they did not pair with each other as usual, but one of them paired instead with the Y, leaving the other X free to go either to the opposite or to the same pole as the first X). Thus some of the eggs in which the above process has occurred come to contain two X-chromosomes, whereas normal eggs contain only one. Now, it is found that in those eggs which receive both X's, no interchange has taken place between them, whereas in the eggs containing one X, interchange has taken place about as often as usual. Hence interchange is connected with whether or not the Y allows the two X's to unite and separate properly, *i. e.*, interchange seems to be a result of the way in which chromosomes pair and separate during synapsis, and, as we have seen, if interchange occurs at this period, it must be by crossing-over.

B. The Correspondence between Separation Frequencies and Chromosome Lengths

In the present section still another possible test will be given of the conclusions arrived at by Morgan, that the factors are in line in the chromosomes, and that the order in which they lie determines in a general way the relative frequencies with which they separate from one another. And it has just been explained that evidence for these ideas is also evidence for crossing-over: that if the diagrams do represent the chromosomes and show the factors in their real order, then the facts of linkage demonstrate that, during synapsis, whole sections of the chromosomes change places at once, *i. e.*, cross-over.

The second test of the validity of the chromosome diagrams is as follows: If the order of the factors shown by their linkage relations, and represented in the diagrams, is their real order in the chromosomes, then it would be

possible, by adding together the frequencies of separation between all *adjoining* factors, to obtain the *total* frequency of crossing-over in the chromosome. This total frequency would be represented in the diagrams by the total length of the latter, since it is always the per cent. of separations between the most closely adjoining factors which is chosen to determine the number of units of length in any region of the diagram. Now, the total frequency of crossing-over in a chromosome ought, we should expect, to be determined by the length of that chromosome. Accordingly, we should expect to find differences between the total frequencies of interchange (or the diagram lengths) of the different groups of factors exactly paralleling the size differences existing between the chromosomes themselves. It will be seen, however, that such an expectation assumes also (1) that crossing-over occurs with equal frequency in all parts of a chromosome, and in equal parts of different chromosomes, and (2) that the factors available for working out the total frequency of interchange do not lie in any one limited region of the chromosome, but are more or less scattered, some of them lying near each end. A negative result from our test, then, might merely mean that one of these two assumptions was incorrect, and this would not disprove any essential point in the theory of crossing-over previously outlined. On the other hand, a positive result would seem to be too much of a coincidence to happen by mere chance, and so would seem to prove the correctness both of our main theory and of the two latter points.

In regard to the size relations existing among the chromosomes themselves, as determined by cytological observations, the work of Stevens (13), taken in connection with the later work of Metz (5), and of Bridges (2), shows that there are four pairs of chromosomes in *Drosophila:* a pair of moderately long sex chromosomes, two pairs of very long "autosomes," and one pair of minute "autosomes."

We may next consider the lengths of the genetic groups,

as determined by their "total frequencies of interchange." The length of the first, or sex-linked, group of factors has been found to be about 66 units; a unit, it will be recalled, is a section of the chromosome of such length that breakage occurs within it, on the average, one time in a hundred cases. The evidence then shows that, in a hundred cases, the first group breaks 66 *times.* This does not mean that it breaks in as many as 66 *cases* out of 100, for it may break two, or, very rarely, even three times, coincidently (at different points along the chromosome) in the same case ("double or triple crossing-over"). As previously explained, when two breaks thus occur coincidentally, the extremes of the chromosome come to lie on the same side, and so a factor at one end of the first group does not separate nearly 66 times in a hundred from a factor at the other end; owing to these coincident breaks it really separates in only about 45 per cent. of cases. The number 66 is consequently not obtained by merely determining the frequency of separation from each other of the two most frequently separating factors, but, as mentioned above, it must be derived by adding together the frequencies of all the smallest parts of the chain (frequencies of AB + BC + CD, etc.). In the case of the first group, the determination of this "total-length" has been accomplished by the combined efforts of a large number of people, although by the work of Morgan, Sturtevant and Bridges particularly.

Group II has a much greater length. It is probably over a hundred units long, and is certainly over 90. This result has been obtained principally by the work of Bridges and Sturtevant, although, as before, others have helped very materially. Mention must here be made of the fact that Sturtevant has discovered in this group specific mutant factors which, when *heterozygous,* lower the frequency of separation in certain regions of the group very much, although the order in which the factors are linked is not changed (16). The variation certainly proves, however, that (if the groups represent the chromosomes)

then, under certain special conditions of heterozygosis, different regions of the same chromosome may differ in regard to the frequency of crossing-over within them, for different regions were not affected in the same way by these factors; it also shows that equal lengths of different chromosomes may have different frequencies of crossing-over, for these factors affected only group II appreciably.

In group III, crosses involving several combinations of different factors have been made by Sturtevant, Bridges and Dexter, but the order of none of the factors has until recently been worked out by them nor has any consistent general scheme been attempted. The information has, in fact, not been adequate for this purpose, and much confusion has also arisen on account of the great linkage variation in this group, which seemed to occur very frequently. Sturtevant, who, as stated in section I, discovered the first case of linkage in the third group—namely, that between pink and ebony—had determined the initial positions of these factors, placing them at about 4 units apart, and next Bridges, who had found kidney (eye), had determined its position at about 15 units from pink, though he did not determine the relation between kidney and ebony. As a matter of fact, however, the kidney determination had been made with flies in which there was a greater frequency of crossing-over than in the experiments of Sturtevant, and, as will appear later, in any given experiment kidney is really nearer to pink than is ebony. From time to time after this other mutants were discovered (peach, Bridges, May, 1913; sepia, Wallace, May, 1913; spineless, Bridges, May, 1913; deformed eye, E. Cattell, 1913; band, Morgan, 1913; rough, Muller, June, 1913; sooty, Sturtevant, Oct. 1913; spread, Dexter, Nov. 1913; dichæte, Bridges, July, 1915), and the fact that these mutants were members of the third chromosome group was determined (peach, sepia, spineless, band and dichæte by Bridges; deformed by Cattell; rough by Muller; and sooty by Sturtevant). The author mean-

while undertook experiments with a view to determining the order of these factors, their frequencies of separation, and the manner in which these frequencies vary, and also sought to correlate with the results the data previously obtained.

It has developed from this work that group III is of the same "order of magnitude" as the first and second. This is the result required by the cytological facts. To complete the parallel, it should be found that the third group is longer than the first and, in fact, of just about the same length as the second group. Whether this is true can not yet be stated definitely, but the results indicate that it is. It is certain that the length of the group of factors dealt with is at least 55, but another estimate, which, for reasons given below, would seem more probable, gives the length as over 100. It should also be borne in mind that not as many factors have as yet been worked with in this group as in the other two, and it may well be that other factors will be found to lie beyond any of the twelve which have so far been approximately "placed." Thus, even if 55 should be the normal value for the factors *dealt with*, the whole group may very well be considerably longer. In the first and second groups, factors lying well beyond all the others were discovered after the positions of more than a dozen had already been determined.

The reason for the uncertainty in regard to the total frequency of separation among those factors which have been worked with is to be found in the linkage variation. Sturtevant had discovered that certain races, containing the mutant factor for ebony body color, gave extremely low frequencies of separation; that is, ebony flies, when crossed to those with pink eyes (pink is also in group III), gave a hybrid in whose germ cells very little recombination between pink and ebony occurred. Other races (*e. g.*, those containing sooty, an allelomorph of ebony), when crossed to pink, gave higher values. He therefore concluded that the ebony flies contain a factor (let us call

it C) which reduces the frequency of separation, and which is dominant, since it produces an effect in the hybrid. I have found that two other races of flies, one having the factor for spread wings (also in group III), and the other showing no "visible" mutant factors, also contain C, as they behave in the same way as ebony. However, the hybrids produced when these races are crossed with ebony give high frequencies of recombination again! This result shows that, as in group II, these races do not really contain a factor which normally reduces separation frequency, for, when both homologous groups of an individual contain the factor C—*i. e.,* when it is homozygous—the frequency of separation is high again. (This also explains an irregularity observed by Dexter, who obtained a high frequency of separation in a cross involving ebony flies.)

It happens, however, that these high separation frequencies obtained when C is homozygous are even higher than those occurring in crosses not involving C at all, and so presumably homozygous for its allelomorph, c. By analogy with Sturtevant's findings in the second group, this would mean that in most crosses hitherto made not involving C there has nevertheless been another factor heterozygous, which has a similar, but lesser, effect on the regions of the group studied. Some support for this interpretation is found in the fact that occasionally higher frequencies are obtained in these crosses not involving C, which appear to overstep the limits of chance variation. The evidence thus far secured on groups II and III points to the conclusion that the highest frequency obtained is that which should be regarded as the normal value, and that very marked departures from this, which affect only a particular group, are generally due to heterozygosis in special factors of that group. If it should be found that marked differences affecting the total frequencies of particular groups do occur, in cases where the flies are homozygous for whatever factors influencing linkage they may contain, we might naturally expect that

such variations would have gradually accumulated in the course of evolution, until no correspondence remained between the relative lengths of the chromosomes and the total separation frequencies. But the parallel which does exist between the observed chromosome lengths and the usual (homozygous) total frequencies, would seem too close to be meaningless, and so we should be led to believe that for some reason marked variations in the frequencies of particular groups, even though they may be possible, do not generally persist; in other words, the frequencies seem usually to stay at least roughly proportional to the actual chromosome lengths, and to furnish another verification of the theory of crossing-over. Further evidence of this will be met with when we consider group IV.

As the data whereby the positions of the factors and the total frequency of separation have been determined in group III, have not hitherto been published, it may be of interest to present some of them here. In order to obtain data on as many combinations of factors as possible in the same cross, so that the linkage values between the different factors would be comparable, I have endeavored to make up, by cross-breeding, stocks containing six or seven mutant factors in group III at the same time.

Since on account of the baffling linkage variation, the order of these factors could not well be determined by combining the results of separate experiments each of which dealt with only two factors at a time, it required a great many trial matings before such multiple stocks could be made up, as of course the crosses have to be made in a certain precise order, to secure a combination of many linked factors. To obtain stock ABC, for example, it would not suffice to make up AC and then mate it to B, for it would then require two coincident recombinations (which might never occur) to secure ABC. Moreover, as a first step it had been decided to get combinations of ebony with various factors, and very much time was lost in this attempt, as it was not then known that

when ebony is crossed to most other stocks recombination of the factors is nearly impossible. A stock has finally been obtained, however, combining the following characters belonging to group III: sepia eye color, dichæte (bristles, and wing), pink eye color, spineless body, kidney eye, sooty body color, and rough eye. Data have not yet been secured with this final stock, but the following experiment, in which most of these factors were involved, may be regarded as typical of crosses not involving factor "C," and consequently giving moderately high frequencies of separation. Hybrid females from a cross of sepia flies with flies containing dichæte, spineless, kidney, sooty, and rough, were backcrossed to the quintuple recessive stock—sepia, spineless, kidney, sooty, rough (dichæte being a dominant). The count of offspring is shown below. The classification as regards kidney has not been given, as this character can not be distinguished with certainty in eyes which are also rough.

No Separation

se	dic sps so r
131	109

Separation Occurring at a Single Point

1. Between positions of se and dic		2. Between dic and sps		3. Between sps and so		4. Between so and r	
se dic sps so r	normal	se sps so r	dic	se so r	sps dic	se r	sps dic so
9	16	20	15	23	19	40	28

Separations Occurring Coincidently at Two Points

1; 2. Between se and dic; dic and sps		1; 3. Between se and dic; sps and so		1; 4. Between se and dic; so and r	
se dic	sps so r	se dic sps	so r	se dic sps so	r
1	2	1	2	3	5

2; 3. Between dic and sps; sps and so		2; 4. Between dic and sps; so and r		3; 4. Between sps and so; so and r	
se sps	dic so r	se sps so	dic r	se so	dic sps r
0	0	2	3	1	1

In addition, 1 sooty spineless fly appeared, which must have resulted from a separation at three points coincidently ("triple crossing-over"), namely, between dic and sps, sps and so, so and r.

The above classification of the flies, in respect to where separation of factors occurred, is based on the assumption that the factors are linked in the order: se-dic-sps-so-r, as on any other arrangement the above results would show many more coincident separations between certain factors, than single separations. The reader may convince himself of this by working out the numbers of the different kinds of separations on any other scheme. We may say, then, that the above results prove that the factors are linked in the order just given. Turning to the individual separation frequencies, it will be seen that se and dic separated 25 times when there was no other point of separation, and 14 times when there was a coincident separation, *i. e.,* 39 times in all. As there was a total of 432 flies this means that these factors separated in 9 per cent. of cases, *i. e.,* are 9 units apart. In a similar way the results show that the distance between dic and sps is 10, between sps and so is 11, and between so and r 19.5, giving a total distance between se and r of $9 + 10 + 11 + 19.5 = 49.5$, which agrees well with the value 55, obtained by combining all the records of crosses of this general sort.

Factors Involved	"Distance" Between Them	Number of Flies on which Result is Based
Sepia dichæte	9.7	624
Dichæte spineless	11.0	683
Sepia spineless	23.4	1,014
Spineless sooty	11.7	1,198
Sooty rough	19.6	1,097
Pink spineless	8.5	825
Pink kidney	11.1	963
Kidney sooty	9.6	885
Pink sooty	19.9	1,566
Deformed pink	3.0	166
Kidney band	8.4	237

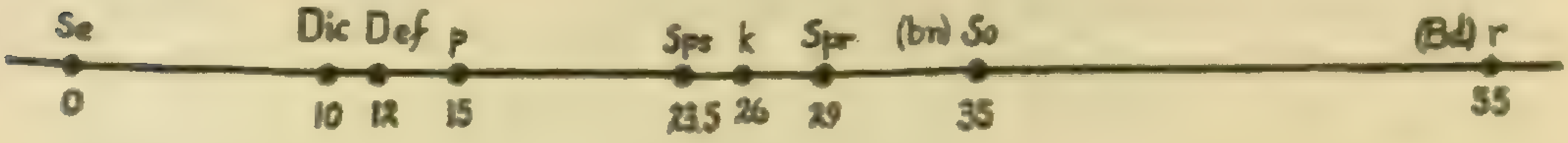

FIG. 5.

The following summary of "distances" between various factors of the third group (considered two at a time)

was obtained by averaging together the results of various experiments not involving C, in which I had followed the inheritance of both of the factors listed at the same time. Many of the data listed in separate lines are of course derived from the same experiment, as in most experiments more than two pairs of factors were followed. It should also be added that in one of the cases below (se sps), the distance was slightly lengthened to allow for coincident separations, the approximate proportion of which was known, but the actual number of which had not been determined in most of the crosses.

Having determined the order of the factors, these results may now be combined, in order to obtain a series of values based upon as many data as possible, and to construct a diagram of the group. The diagram so made is shown in Fig. 5. The numbers underneath the symbols of the factors represent the "distance" of the latter from sepia, which, as it lies at one end of the group, is used as a common point of reference. Although the distances shown will undoubtedly be subject to revision, the order of all the factors shown, except deformed, band, and beaded, is certain. Deformed (eye) is surely between sepia and pink, but it is not yet quite certain that it is to the right of dichæte; band (thorax) is near sooty, but on which side is not known; beaded (wing) is very near rough, but it has not been established whether it comes before or after it (a count of 50 flies showed no crossing-over between them). It was Sturtevant who first determined the position of beaded (found by Morgan, May '10) with reference to this series (to the right of sooty), and Bridges who first determined that dichæte lies between sepia and pink (about 4 to the left of pink). The data listed merely confirm these findings, so far as these two factors are concerned. And it may here be repeated that numerous other crosses of factors in this group have also been made by these investigators, although the crosses have not been of a sort to show the arrangement of the factors studied.

We may next consider the disposition of the factors of group III in a diagram based upon data from flies heterozygous for C. The separation frequencies which I have obtained are given below:

Factors	Per Cent. of Separations	Number of Flies
Sepia spineless	20.9	527
Spineless kidney	0.0	527
Pink kidney	1.5	868
Kidney sooty (or ebony)	0.2	674
Sooty (or ebony) rough	0.0	843
Kidney rough	0.1	1,211

A diagram based upon these data would show sepia at 0, pink at 19.5, spineless and kidney at 20.9, sooty and rough at 30, and the total length would thus be 30. The tenth of a unit of distance between kidney and sooty is based upon one fly, in which separation had taken place between these factors. Tests of the fly (which was a sooty rough, resulting from a backcross of a female containing p sps k so r from one parent and spread C from the other) showed that the factor C had remained with spread, and that no recombination had taken place between the positions of spread and sooty. This one fly, therefore, proved that both spread and C were to the right of kidney. The factor C is thus seen to lie right in the heart of the region where it exerts its maximum effect, as Sturtevant has also found in the case of the similar factors in group II.

Sturtevant has obtained slightly higher frequencies of separation between pink and ebony in some of his crosses heterozygous for C. The lower value here recorded may then be due to the flies being heterozygous for another factor besides C which disturbs separation frequency, and which is also met with in the crosses not involving C.

The following values have been obtained in crosses in which the factor C was homozygous:

Factors	Per Cent. of Separations	Number of Flies
Sepia pink	27.2	136
Pink ebony	48.0	290

Besides this, it is found that spread is about two thirds of the way between pink and ebony. Ebony, it will be remembered, is an allelomorph of sooty, and therefore occupies the same position. As no recombination has yet occurred between C and rough in flies heterozygous for C, it has not been possible to obtain these factors together and so, in crosses homozygous for C, the linkage of rough has not yet been discovered. The length of the group between sepia and ebony is 75 in these flies, as will be seen from the above data. Although these figures are based on a relatively small number of flies, the difference between this and the shorter value (35) found in flies not containing C is marked enough to be significant, especially since it occurred in various crosses of this sort. If the distance between sooty and rough is expanded in the same way, the group would have a length of much over 100. If, however, this distance is of the same length as in flies without C, the total length would be 95. The reasons have been given which incline us to the opinion that these values obtained in crosses homozygous for C may represent the "normal" figures for this group rather than those obtained in the experiments earlier cited. Further investigation of this point, however, is being undertaken.

Group IV corresponds with the pair of small chromosomes in that it contains so few factors. For this reason, the author, in his account of the inheritance of bent wing, in 1914, said:

> It also seems probable that when other mutations are discovered in the fourth group, the genes in which they occur will be found to be linked strongly to the gene for bent wings, since the fourth chromosome is probably the small one, and so any genes in it must lie near together.

One other mutant factor, "eyeless," has since been found, by Miss Hoge, to lie in this group. But although Miss Hoge has made numerous attempts (3) to combine eye-

less and bent, no recombinations between them have so far been obtainable. Group IV, therefore, forms a marked contrast to all the other groups as regards the frequency of separation within it, and this result is the more striking, not only because it shows that there is a group of factors corresponding in separation frequency to the pair of short chromosomes, but also because it happens that this group is the same one as that which had previously been identified with the pair of short chromosomes by reason of the fewness of the mutant factors discovered in it.

It is therefore evident, not only that the relative sizes of the chromosomes are in a general way like the separation frequencies of the groups, but also that where there is evidence from another source indicating in which chromosome a certain group lies, this is the very one to which the group corresponds by its total frequency of separation. It has been shown that this is true in the case of the fourth group. In the case of the first group, the sex-linked inheritance of the latter connects it with the X-chromosome, and since this is the moderately long chromosome, it is just this one with which group I would be identified by its frequency of separation. The other two groups, both of which are long—one certainly very long, and the other probably so—are thus left to correspond with the remaining chromosomes, both of which are very long and indistinguishable in appearance.

In the remainder of this article, therefore, the word "chromosome" will be used instead of "group" and "crossing-over" instead of "separation of linked factors."

(To be continued)

INDIVIDUAL DIFFERENCES AND FAMILY RESEMBLANCES IN ANIMAL BEHAVIOR

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In experimental work on animal behavior, but little attention has been paid to individual differences, and practically none to family resemblances.[1] In studying the inheritance of conduct in man, experimental methods can not be used. Students of eugenics depend on observations difficult to verify. In the work here described an attempt has been made to apply the methods of genetics to the study of conduct. Such work was begun by Professor J. McKeen Cattell some fifteen years ago, but the results obtained by him and his students were not published, and the problem has been given to me.[2]

The plan of the experiment is to measure individual differences in behavior, to determine the extent to which the animal which departs from the average in one direction will depart in others, to measure the resemblances in families and in lines of descent, and to determine the degree to which kinds of conduct can be established in family lines by selection. It is evident that such a problem can be solved only by many years of work and with the facilities of a research institution. In the present paper there are described the individual differences and family resemblances of 90 mice, as determined by the time required to find their way through a maze. The same mice have been tested in other ways, and further experiments are now in progress with the F^5 and F^6 generations.

[1] Basset has recently published an article on "Habit Formation in a Strain of White Rats with Less than Normal Brain Weight." *Behavior Monograph Series*, No. 9, 1914. Macdowell in *Science* for November, 12, 1915, gives a brief abstract of work on "Parental Alcoholism and Mental Ability. A Comparative Study of Habit Formation in the White Rat."

[2] The greater part of the work presented in this paper was done at Columbia University, the results being used for a master's thesis.

A maze, designed by Professor Cattell, was used, the plan of which is shown in Fig. 1. The animal has in the

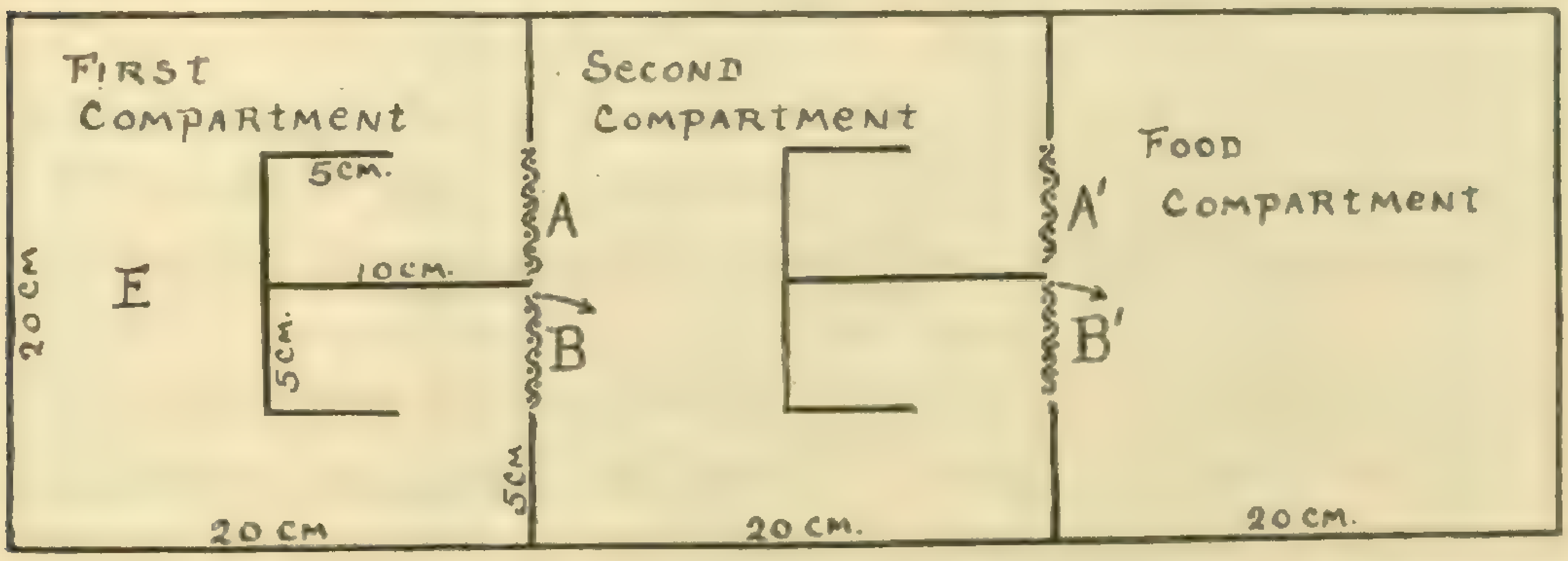

FIG. 1. Diagram of Maze. *A* and *A′* = closed wire gates; *B* and *B′* = open wire gates; *E* = entrance from above.

first compartment the alternative between two gates, one of which can be pushed open while the other is locked, and then it has the same alternative in the second compartment. When it takes the correct way in both compartments, it finds itself in the food compartment. The path that the animal must follow can be altered by changing the gates which are locked. "Unit construction" is used in the dimensions, which are adjusted to the size of the animal, and in the fact that any desired number of standard units can be added.

Preliminary tests were made with albino rats, but these were given up for mice, which are more active and more easily handled. The mice were given one trial each day, and were tested at as nearly the same time as possible. Light was found to play but a minor rôle in the tests, daylight and artificial light serving equally well. At the outset the age of the mice when first tested was not always known, but later when the various litters were obtained the young mice were tested at or about four weeks old.

The mice were rewarded for a successful trial by a mixed diet of milk, bread, oatmeal and sometimes meat. They always had a little dry bread in their cages. Besides satisfying their hunger, the mice had the additional reward of a place for exercise and the companionship of the mice that had just been tested. The order of the tests was

varied day by day. In case the way through the maze was not found in 360 sec. the animal was removed and tested again the following day. 360 sec. is thus the maximum record for a single trial.

Seventeen trials were made with each individual. This was a desirable number for two reasons: first, because this number was sufficient for the average mouse to learn the maze, and secondly, because the seventeen trials could be divided into three somewhat homogeneous groups. The first two trials are largely affected by chance, so, although given here for completeness, they are not averaged in the final ratings for each individual. The second group of five trials represents the period of more rapid learning, the third group of ten trials the results when the learning is slow or completed. In this paper the averages of the last fifteen trials are used as the index of performance. For some purposes the last ten or the last five trials might be preferable. The rate of learning as determined by the relation between the first and last groups may also prove to be of value.

In addition to the time, the number of errors, *i. e.*, the number of cases in which the mouse tried to go through the locked gate, is given in the tables, as this is a measure of the activity of the animal. With only a few exceptions, however, the error and time curves correspond. Consideration of the correlation between error and time, and between performances at the beginning and the close of the trials is postponed until data can be given for a larger number of individuals and the records for other kinds of behavior.

In Tables I to V are given the complete records of the 90 mice tested, grouped in families as described below. The average time is 44 sec. per trial for the last fifteen trials. The distribution of the individuals is shown in Fig. 2. In 41 cases the time was under 20 sec., in 19 cases between 20 and 40 sec., in the remaining 30 cases between 40 and 200 sec. None of the mice failed to learn the maze.

When the experiment started, several colored mice—chocolate, agouti, gray, black and yellow—were tested,

TABLE I

Complete Time and Error Records for the Yellow Family

No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average	No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average
20Y ♀	360	228	58	115	1.6	38Y ♂	360	277	98	157	3.6
21W ♂	247	41	11	21	.2	39Y ♂	360	143	25	64	2.3
22W ♂	234	28	14	19	.4	40Y ♂	360	47	9	21	.8
23AG ♂	280	43	6	19	.5	41Y ♀	210	20	8	12	.5
24AG ♂	182	47	8	21	.9	57Y ♂	360	171	109	130	3.7
25AGW ♀	177	136	26	63	1.7	58Y ♂	357	130	51	77	2.4
26Y ♂	360	206	172	183	3.7	59Y ♂	360	275	98	156	3.3
27Y ♀	201	10	14	13	.5						

In the first column is given the catalogue number, color and sex of the animals. In the second are the time averages (in seconds) for the first two trials; in the third, for the next five trials; in the fourth, the last ten trials, and in the fifth column the average of the two preceding columns. The error average for the last 15 trials is given in the last row of figures. This order is followed in all the subsequent tables.

One day's record has been omitted for mice Nos. 27, 28, 29 and 31 because the poor records for that day were obviously due to a constant error, on account of traveling, etc. These are the only cases where such a condition has occurred.

TABLE II

Complete Records for the White Family

No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average	No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average
12W ♂	360	91	56	67	1.9	103W ♂	108	5	10	8	.5
13W ♀	100	159	37	91	1.4	104W ♂	174	12	13	12	1.0
15W ♀	316	26	17	21	.4	105W ♀	99	12	11	11	.8
48W ♂	156	16	7	10	.5	106W ♀	284	22	12	15	.9
49W ♀	185	75	25	42	1.9	109W ♂	101	69	8	28	.9
50W ♀	246	25	18	20	.7	110W ♂	93	11	7	8	.4
51W ♀	56	13	7	9	.5	111W ♂	67	13	12	12	1.0
52W ♀	169	12	10	11	.9	112W ♂	151	33	8	16	.9
53W ♀	196	77	34	48	1.7	113W ♂	88	11	7	8	.7
65W ♀	285	87	8	35	.9	114W ♀	75	13	5	8	.4
66W ♂	360	306	17	113	1.2	115W ♀	70	13	11	12	1.0
67W ♀	360	173	25	75	1.7	116W ♂	183	13	8	10	.7
76W ♀	222	58	17	31	.9	117W ♂	318	11	4	7	.5
77W ♂	317	162	20	68	1.0	118W ♂	81	14	10	12	1.0
78W ♂	360	186	91	122	2.9	119W ♂	115	40	9	20	1.1
71W ♂	149	121	18	53	.9	120W ♀	118	19	7	11	.5
72W ♀	360	304	30	121	1.7	121W ♀	64	17	9	12	.7
74W ♀	89	29	21	24	1.2	122W ♂	76	84	7	33	.7
86W ♀	84	19	13	15	1.2	123W ♀	66	39	29	32	2.2
87W ♀	58	16	14	15	1.2	124W ♀	25	23	10	14	.9
88W ♀ ♀	86	14	7	9	.8	125W ♂	189	15	7	9	.5
89W ♂	110	16	10	12	.9	126W ♂	90	19	9	12	1.0
91W	252	47	33	38	1.6	127W ♀	72	22	11	15	.9

TABLE III

Complete Records of a Family Consisting Mostly of Yellow Individuals

No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average	No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average
32Y♂	225	109	71	83	1.7	61Ch♂	360	202	30	87	1.0
33Y♂	154	186	42	90	.5	62YW♂	312	90	18	42	1.5
34Y♂	186	88	39	56	1.0	63YW♀	243	130	38	69	1.2
36Y♂	354	55	28	37	.9	64Y♀	360	182	113	136	2.3
37Y♀	137	20	16	17	1.2	68YW♀	112	71	65	67	2.0
54W♀	360	36	34	35	.7	69Y♂	177	41	9	19	.7
55Y♂	360	242	103	150	3.5	70Y♀	234	223	75	124	2.3
56Y♀	360	130	103	112	2.0						

TABLE IV

Complete Records of a Small Family Showing Good Records

No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average	No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average
29W♀	229	9	8	8	1.3	45W♀	142	13	5	8	.5
30Gr♂	58	43	33	36	1.0	46Gr♂	141	9	4	6	.5
44W♀	297	16	7	10	.5	47Bl♂	150	34	29	31	1.0

TABLE V

Complete Records of the Unrelated Individuals

No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average	No.	First 2 Trials	Next 5 Trials	Last 10 Trials	Last 15 Trials	Error Average
1Ch♂	212	48	11	23	1.0	5W♀	74	22	8	12	.6
2Y♀	285	213	103	140	5.1	28W♂	291	38	5	17	1.0
3Y♀	316	55	63	61	3.3	31Gr♀	130	78	15	38	1.0
4W♂	77	18	14	16	.7	85W♂	131	14	9	11	.8

besides a large number of albinos, and among the yellow mice several made poor records. These mice were mated, and they and their offspring compose a group of 27 individuals, whose average time and error record is considerably in excess of the normal for the entire population.[3]

[3] This group of 27 mice was composed (see Tables I, III and V) of Nos. 20 and 26, and their seven offspring; No. 27, the sister of No. 26; a litter of five mice, Nos. 32, 33, 34, 36 and 37 and their ten offspring, and finally two unrelated yellow mice, Nos. 2 and 3, that were used at the beginning of the experiment. The 63 remaining mice of the white group bring the total to 90.

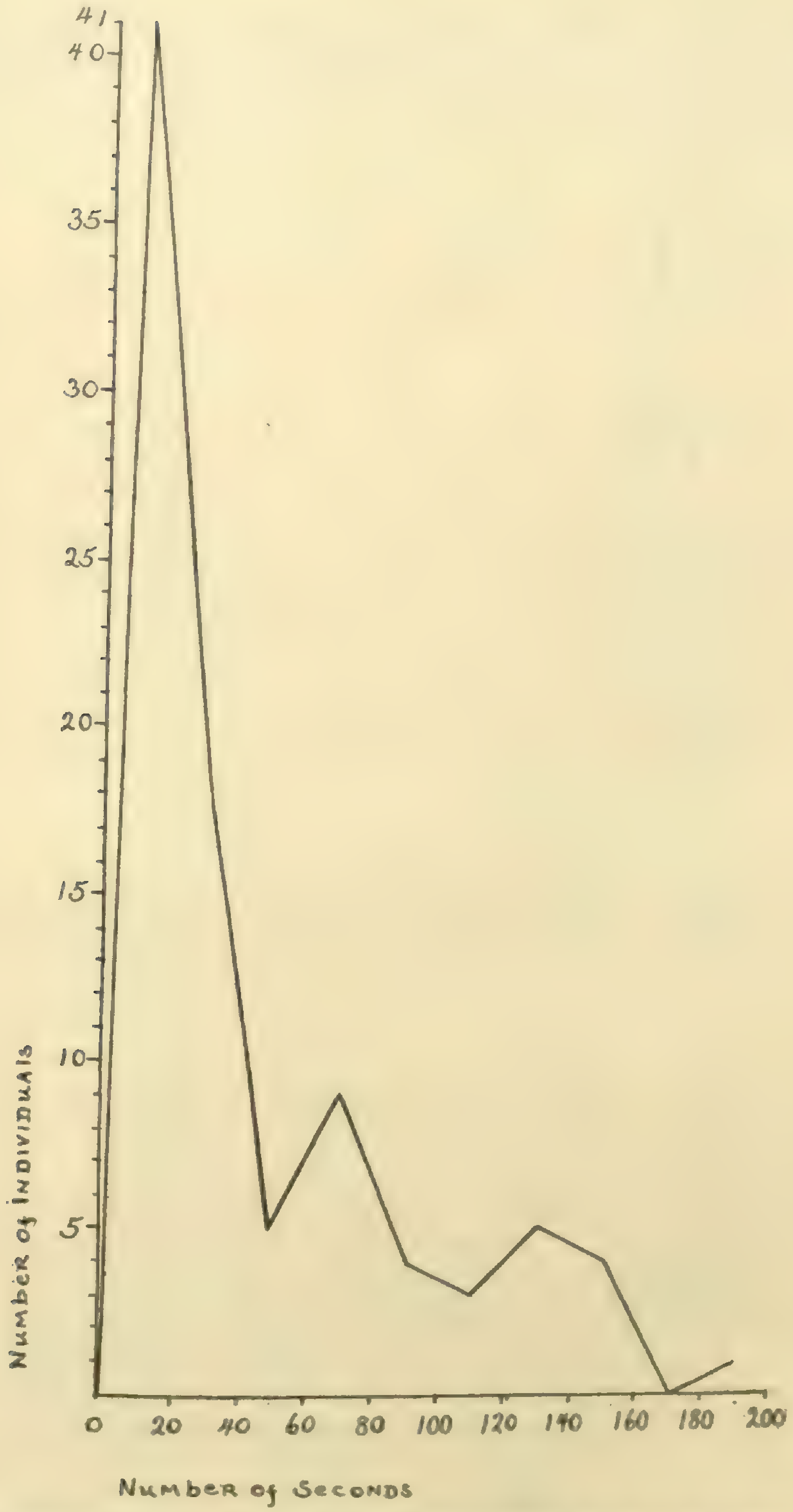

FIG. 2. Total distribution curve for 90 individuals for the last 15 trials.

The yellow group gave an average time of 83 ± 7.0 sec., and an average of 2.0 errors for the last 15 trials. The other mice gave an average time of 27.5 ± 2.0 sec. and .9 error per trial. The yellow mice were thus found to take,

on the average, at least three times as much time and to make twice as many errors as did the white mice. In Fig. 3, the distribution curves of both groups are given, the

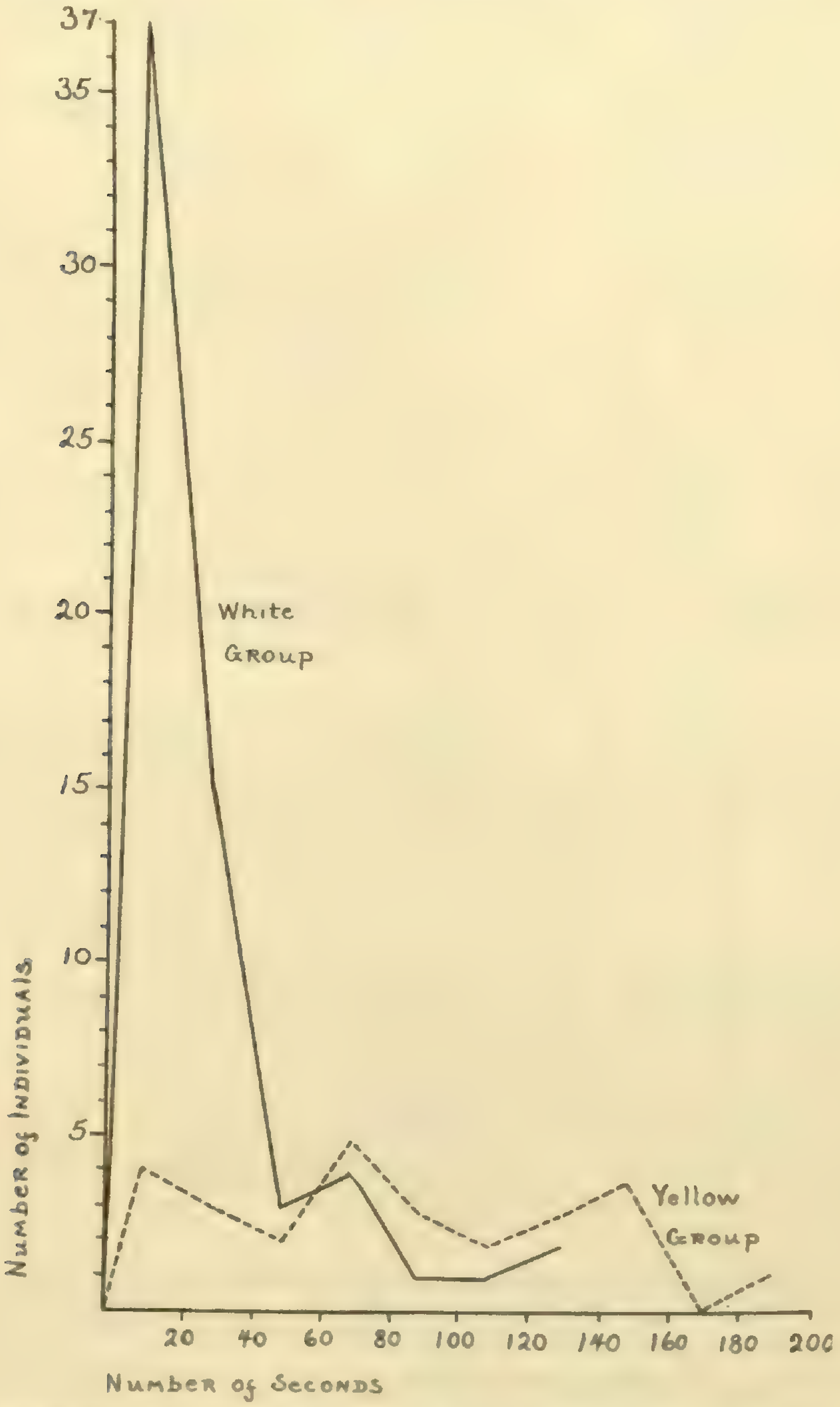

FIG. 3. Distribution curves of yellow and white groups for the last 15 trials.

yellow in a broken and the white in a solid line. The curve for the miscellaneous group is skewed, most of the individuals falling between 0 and 20 sec. The curve for the yellow family is nearly flat, there being about the same number of individuals in each time group.

Fig. 4 gives two average practise curves, one for the group of 63 white and colored mice, and the other for the group of 27 mice that are mostly yellow. The records of

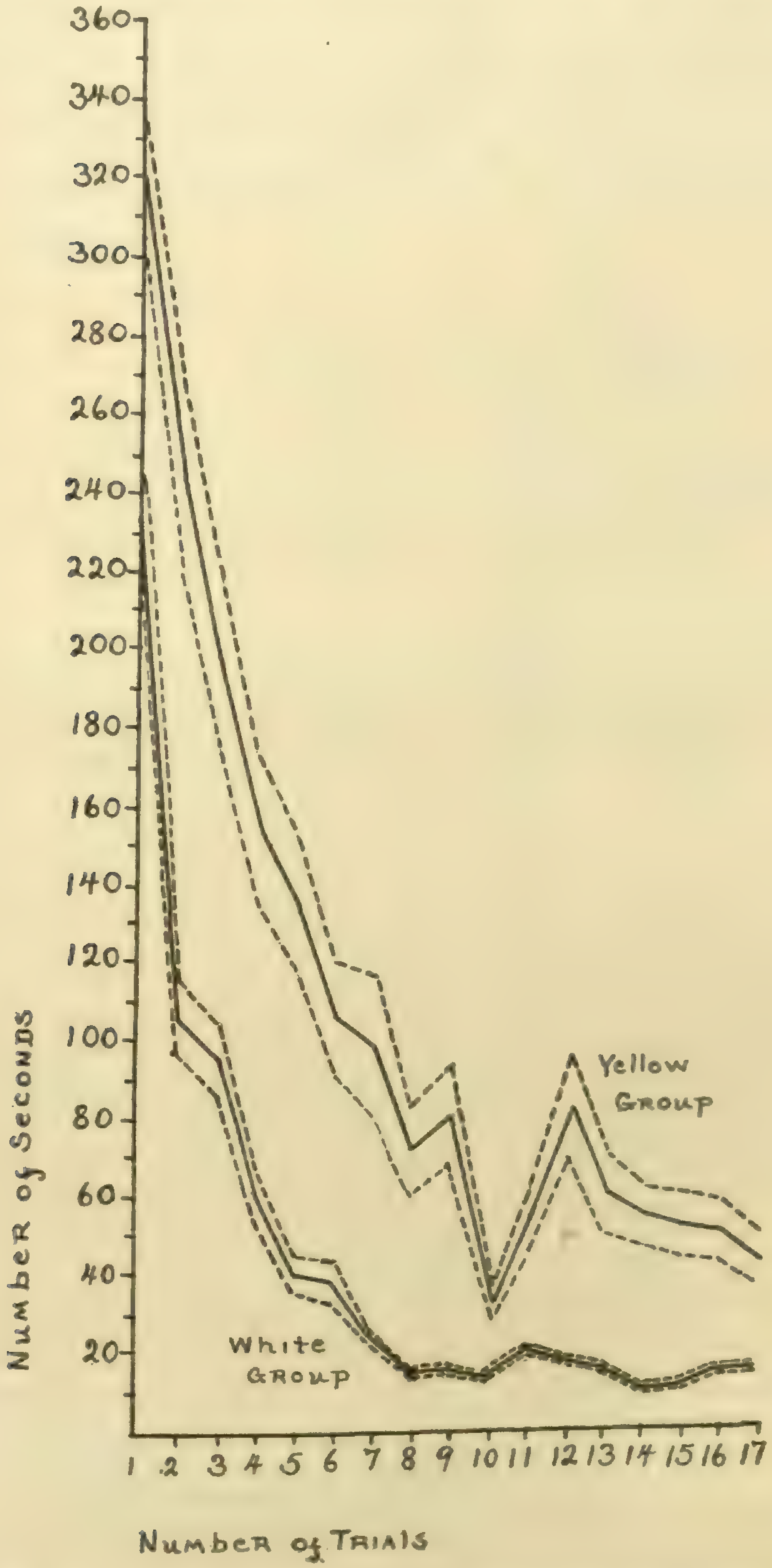

FIG. 4. Average record curves for yellow and white groups.

all the individuals for each successive trial were averaged, and the probable error calculated for each point on the curve. In accordance with a plan proposed by Professor Cattell, the limits of the probable error are shown by broken lines. The chances are even that with a greatly increased number of cases the time would have remained within these limits, and a nearly smooth curve can be drawn within them. A notable exception is the tenth trial with the yellow mice. At this point there is an unusually

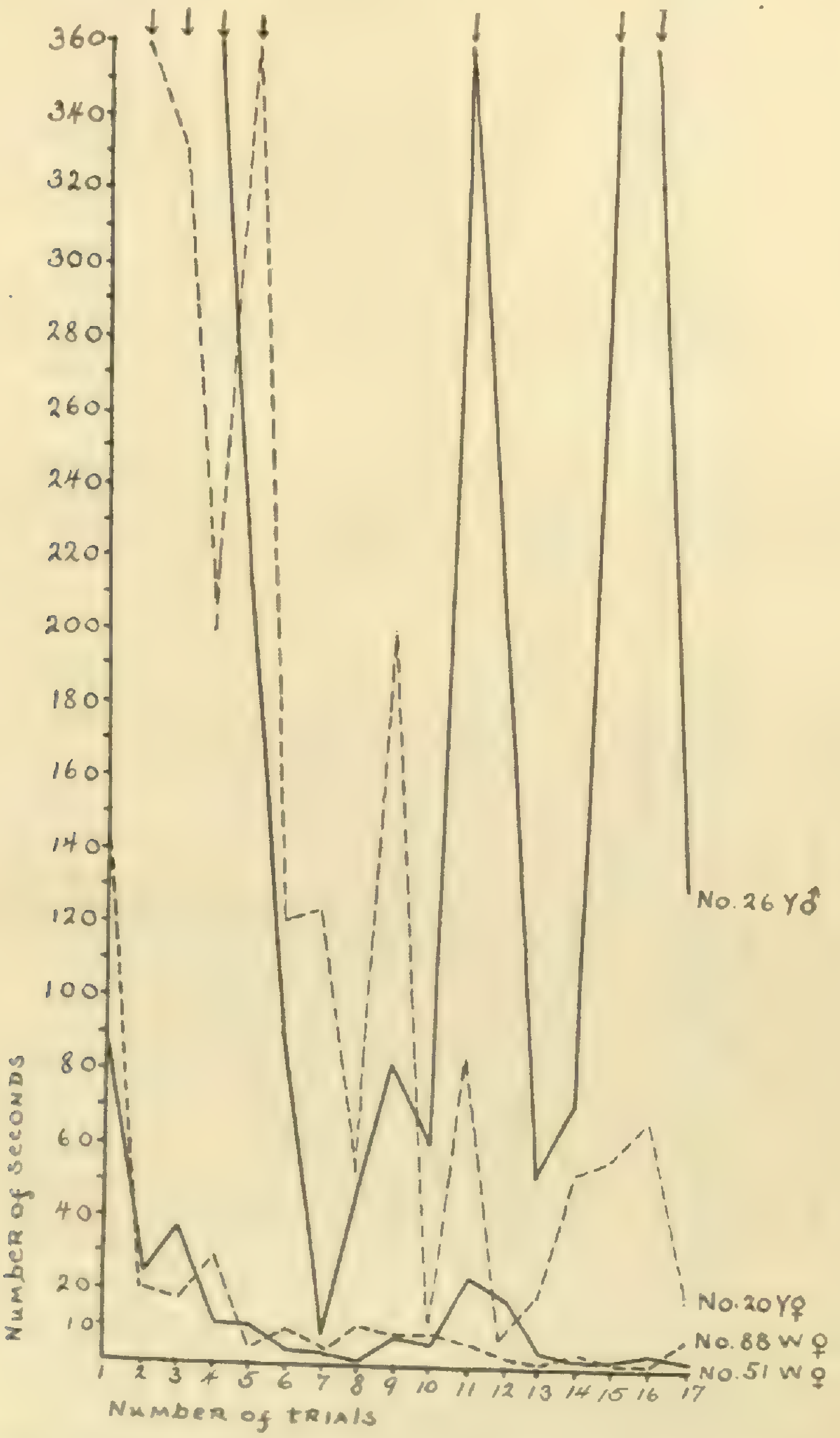

FIG. 5. Daily record curves for two white and two yellow mice.

large number of low records, more than the law of probability would warrant.

In Fig. 5 are given sample practise curves, showing the daily records for two white mice, Nos. 51 and 88, that learned the maze quickly, and for two yellow mice, Nos. 20 and 26, that were slow to learn. The arrows at the highest points indicate that the mouse did not pass through the maze. Thus No. 26 only got through on the fifth trial and failed in the eleventh, fifteenth and sixteenth trials.

The mean variation for the entire group of 90 mice was found to be 35.6. This means that any mouse picked at random from the mixed group would be likely to vary from the average by 35.6 seconds. In order to find whether mice of the same litter varied less than unrelated individuals, the mean variations for each of the 18 families was calculated, and these when weighted for size of family were found to be 20.2. The resemblance in behavior between mice belonging to the same litter was consequently nearly twice as great as between unrelated individuals. This corresponds to a coefficient of correlation in the neighborhood of 0.5 for brothers, as found by Pearson, Thorndike and others.

TABLE VI

AVERAGES FOR SEX DIFFERENCES

No.	Color	Sex	Average Last 15 Trials	Probable Error	Average Number of Errors
32	White, etc.	♂	27.69 Sec.	± 2.9	.9 per trial
31	White, etc.	♀	27.35 Sec.	± 2.9	1.0 "
15	Yellow, etc.	♂	90.0 Sec.	± 9.6	2.0 "
12	Yellow, etc.	♀	75.0 Sec.	±10.3	2.1 "

In Table VI, the males and females are grouped separately, and their average times and errors are given. In both groups of (mainly) white individuals, with 32 males and 31 females, and in the group of (mainly) yellow mice, with 15 males and 12 females, the times for the females are on the average slightly shorter, but the differences fall within the limits of the probable error and indicate that there are no sex differences in this kind of behavior.

We may now take up in more detail the family histories. Fig. 6 gives a graphic representation of matings, from

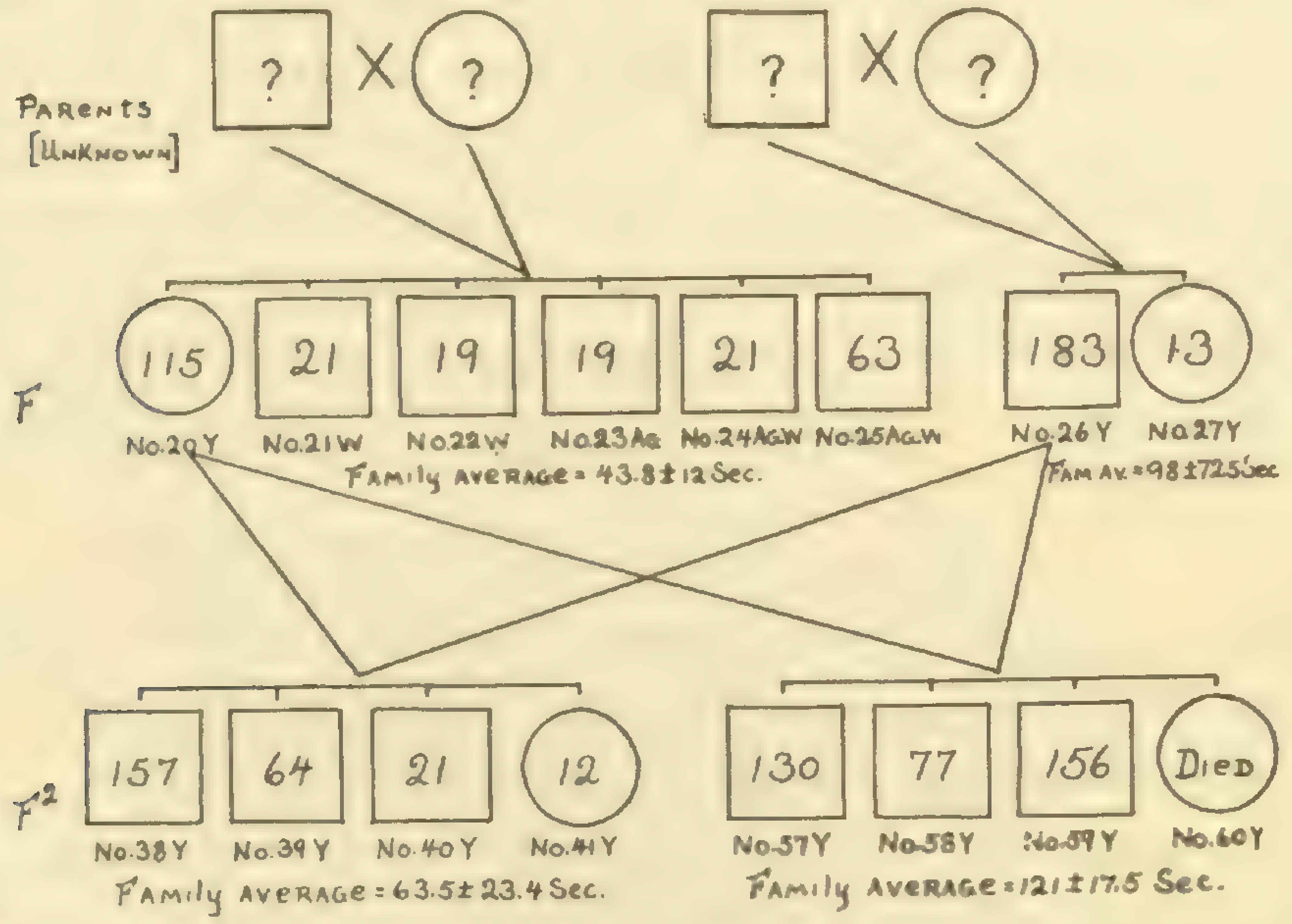

FIG. 6. Descent of yellow family. Squares denote males and circles females. The numbers within the circles and squares represent the average time taken for the last fifteen trials. The manner in which the yellow mice in these experiments have given an unusually large number of yellow offspring has been made the subject of another investigation from a mendelian standpoint.

which there were selected two mice, No. 20 Y♀ and No. 26 Y♂, which made unusually poor records, 115 and 183, respectively, though the other mice in the same litters had good records. The parentage of Nos. 20 and 26 was unknown; they were mated and gave two litters, each composed of three males and one female. Three mice in these two litters gave unusually slow records and made considerably more errors than normal. Two other mice gave poor records; two gave good records, while one died before it was tested. It is unfortunate that both females in these litters died before further offspring could be obtained. Table I gives the complete record of both time and error averages for these mice. It is a question whether or not the selection of parents having poor records tended to produce more than the normal number of offspring slow to learn. Further investigation can alone afford an answer.

The mice whose records are given in Table II are graphically represented in Figs. 7 and 8. They have been

carried down through the sixth generation, and are still being tested. As neither of the mice of the F^4 generation mated with No. 91, the only individual of the F^5 generation, No. 91 was mated successively with four unrelated

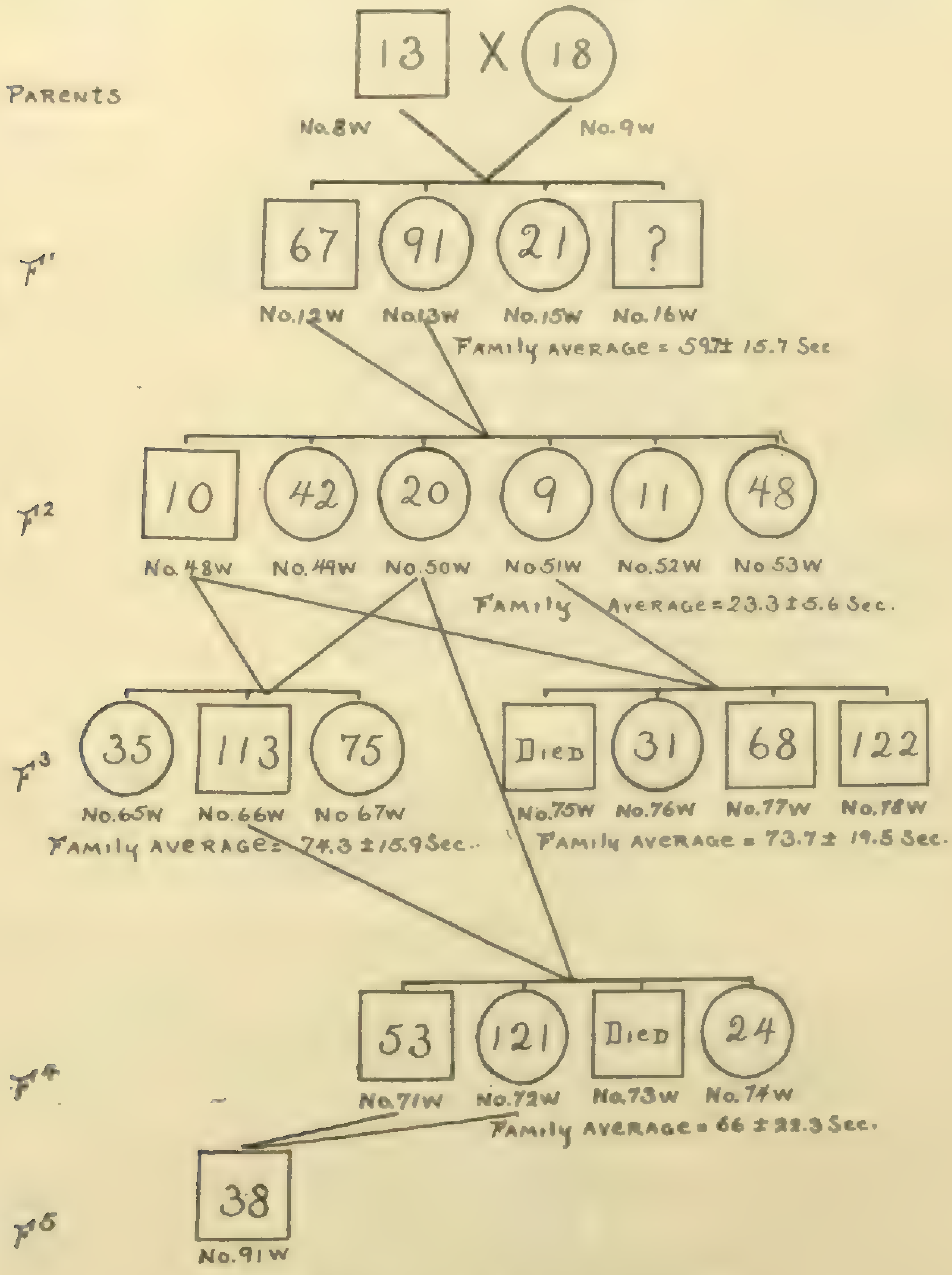

FIG. 7. Descent of a white family.

white females, Nos. 86, 87, 88 and 89. These females had been previously tested and found to give exceptionally good records as indicated in Table II. Twenty-three offspring resulted from these matings. Their records are remarkably uniform and the family averages are the lowest so far obtained. The records of these families are graphically represented in Fig. 8, and here only the continuation of the white family is given showing the F^5 and F^6 generations separately for each individual family. It

is hoped that future offspring may be obtained to continue this strain. The times for the fifteen trials do not always correspond with the times for the last ten trials.

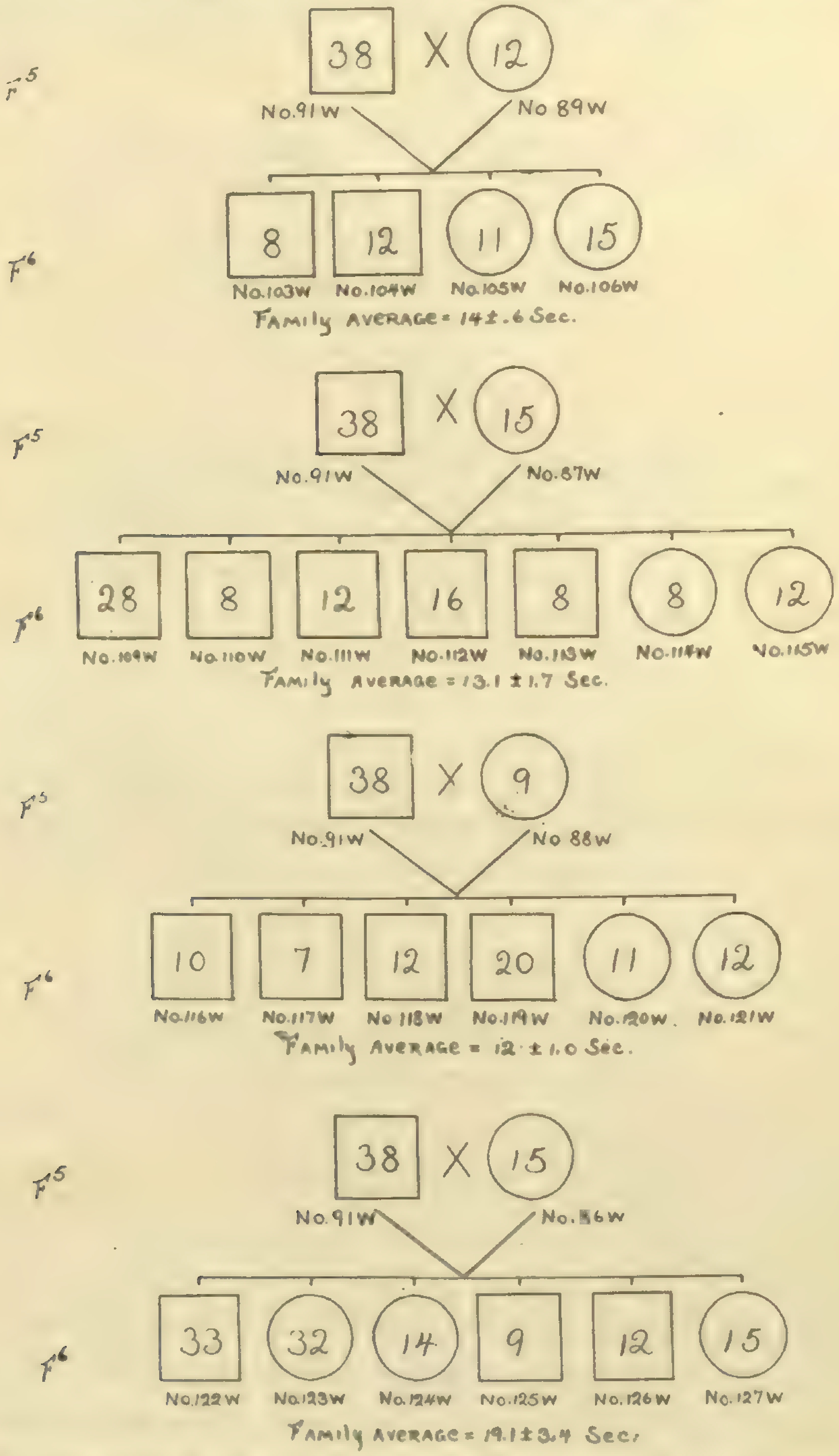

FIG. 8. Continuation of white family. No. 91 mated with four females.

Thus No. 66 has for the fifteen trials an average time of 113 sec., but in the last ten trials reduced the time to 17 sec. The capacity of the mice can only be finally determined after the same individual has been tested by different methods.

Another family, mostly yellow, was derived from a yellow female and an unknown male, probably white. The F^1 from this mating gave a litter of six, Nos. 32 to 37 inclusive. The records of 5 of these (one died) are given in Table III and are graphically represented in Fig. 9. The

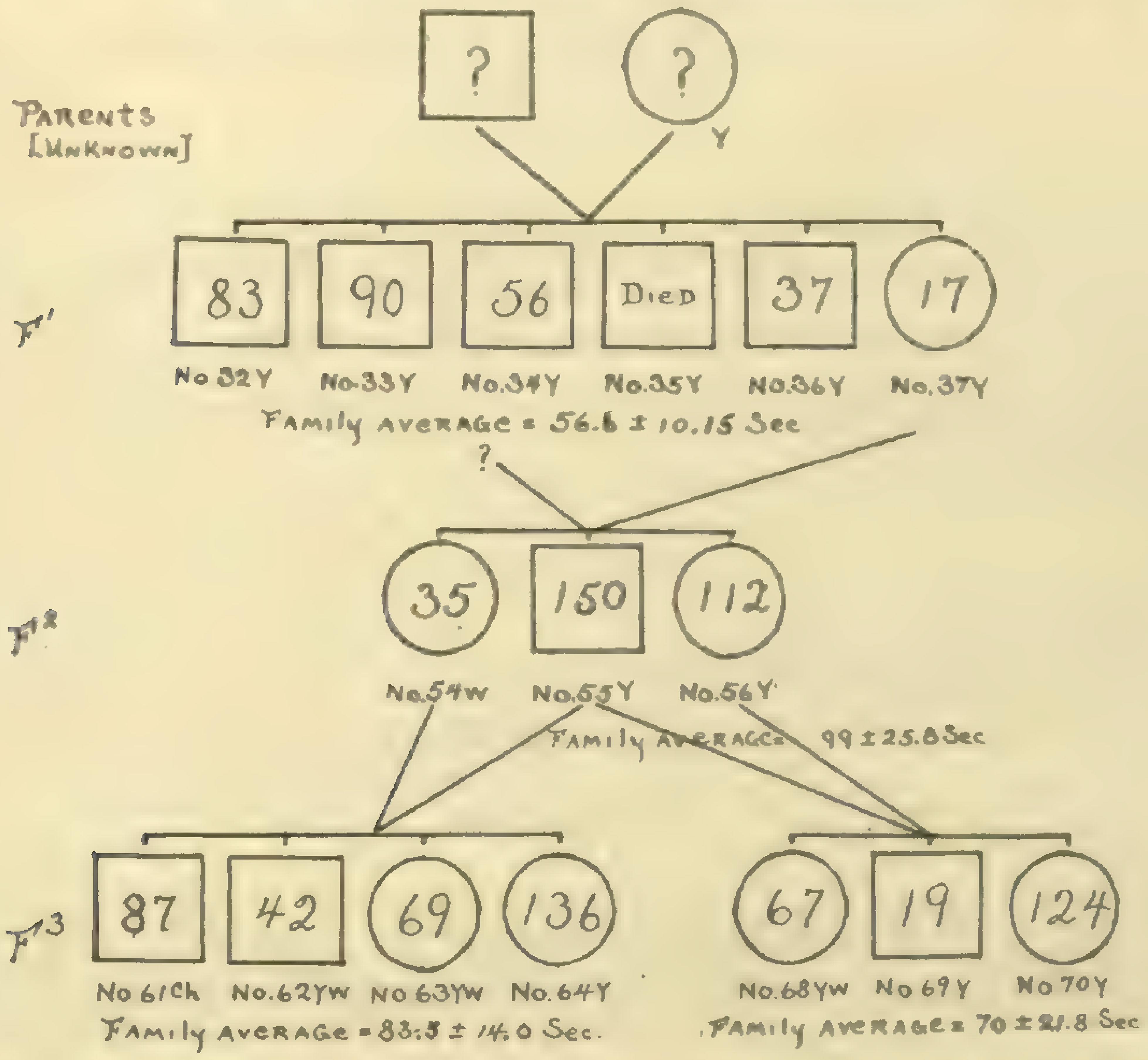

FIG. 9. Descent of a family of mice consisting mostly of yellow individuals.

only female of the litter (No. 37) mated but once, and it is not known with which brother. She bore in the F^2, two females and a male (Nos. 54, 55, 56). Both females of this generation were crossed with their brother and two litters resulted. No. 55 $\times$ 56 gave Nos. 68, 69 and 70 in the F^3, and No. 55 $\times$ 54 gave Nos. 61 to 64 inclusive. From a survey of the complete records of these mice, it is seen that although the F^2 and F^3 generations came from the female, No. 37 (which made the exceptionally low record of 17), still two of her young in the F^2 made poor records, and Nos. 61, 64 and 70 in the following generation did the same.

The records of a family of white and colored mice are given in Table IV and Fig. 10. The two parents and the four offspring all have good records. It is to be regretted that no further litters were obtained from this family.

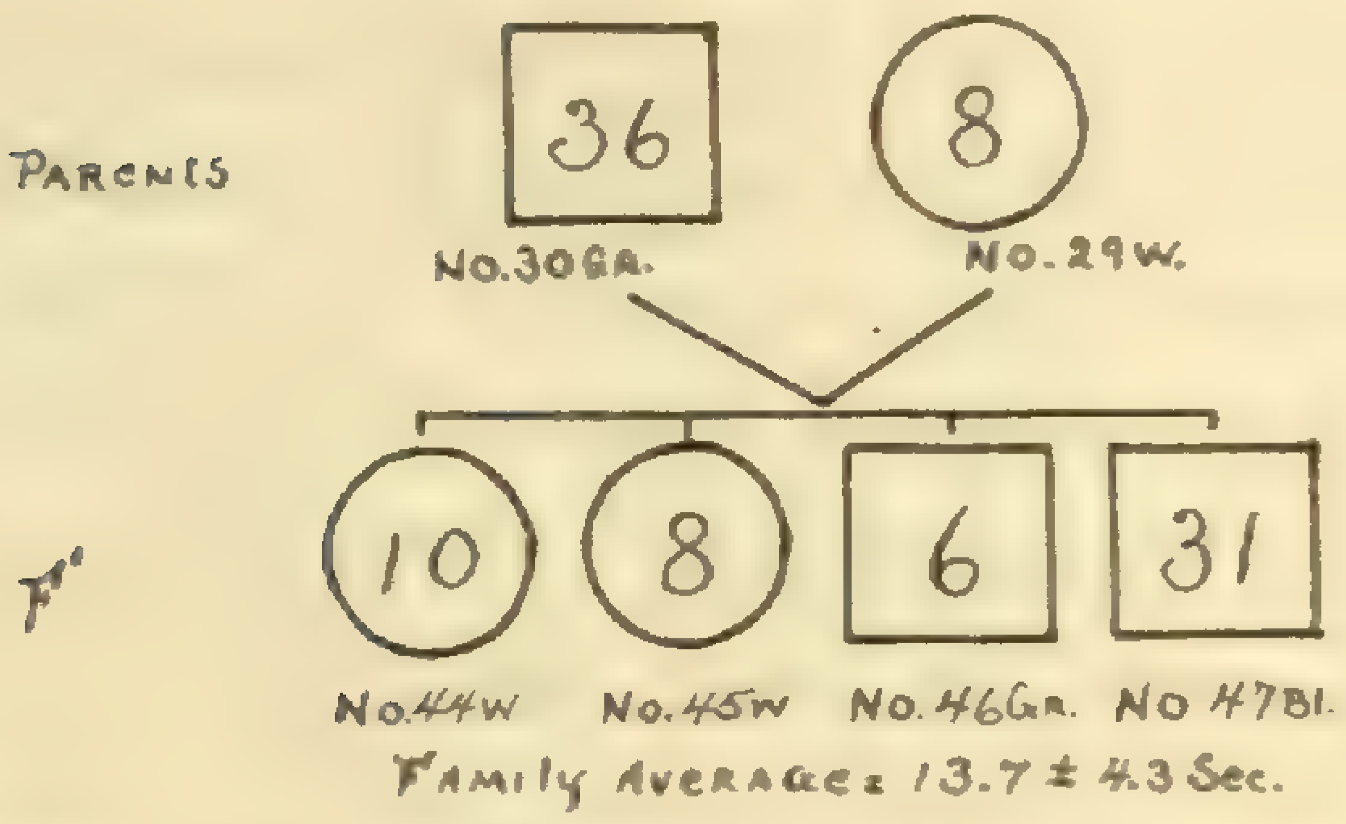

FIG. 10. Descent of a small family of mice showing good records.

In Table V are given the total records for the isolated cases, which complete all the individuals tested. Here again the relative inferiority of the yellow mice may be noted.

SUMMARY

1. Albino and colored mice can be used to advantage for laboratory work on animal behavior.
2. The type of maze used seems well adapted for this kind of work.
3. There is a marked difference in individual behavior.
4. There appears to be a resemblance among individuals of the same litter.
5. There appears to be a considerable difference among different strains.
6. The sex differences, if any, are very slight.

EVOLUTION OF THE CHIN

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In the Smithsonian Report for 1914 is an article by Louis Robinson, M.D., on "The Story of the Chin." Dr. Robinson in this article goes so far as to explain the presence of a chin in human beings as the result of the habit of articulate speech. Quite a different explanation is possible for the existence of this extraordinary feature of our anatomy. I should like to suggest some of the evidence which would seem to indicate that Dr. Robinson's ideas need rather careful review.

By chin is to be understood the projection or point on the under jaw, below the mouth (Fig. 1). The jaws of most vertebrates have no *projection* or prominence in this region.

It will therefore be recognized at the outset that the chin is a very "human" trait. It is a trait that distinguishes man from other living primates; even from his near relatives (compare Figs. 1 and 2). It even sets off

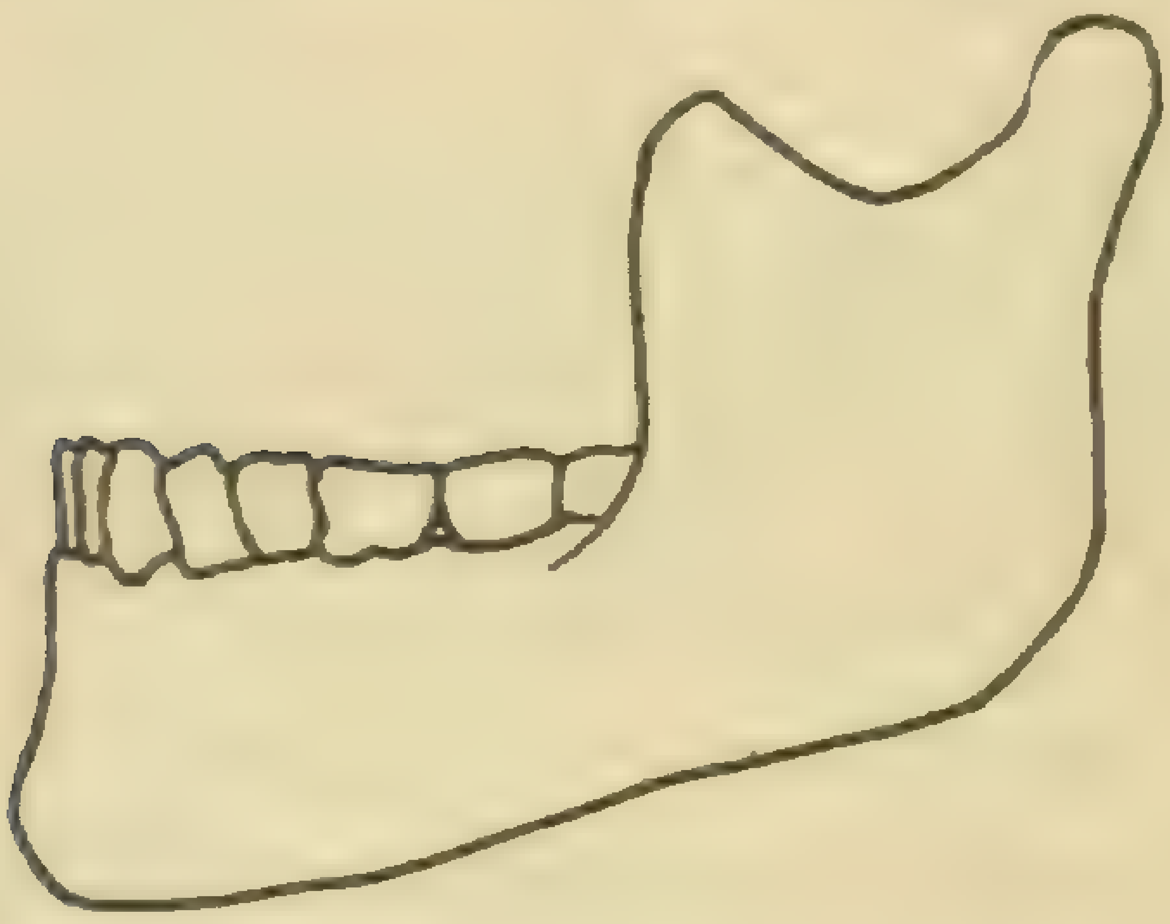

Fig. 1. Recent human lower jaw, showing the so-called "mental" or "chin" prominence.

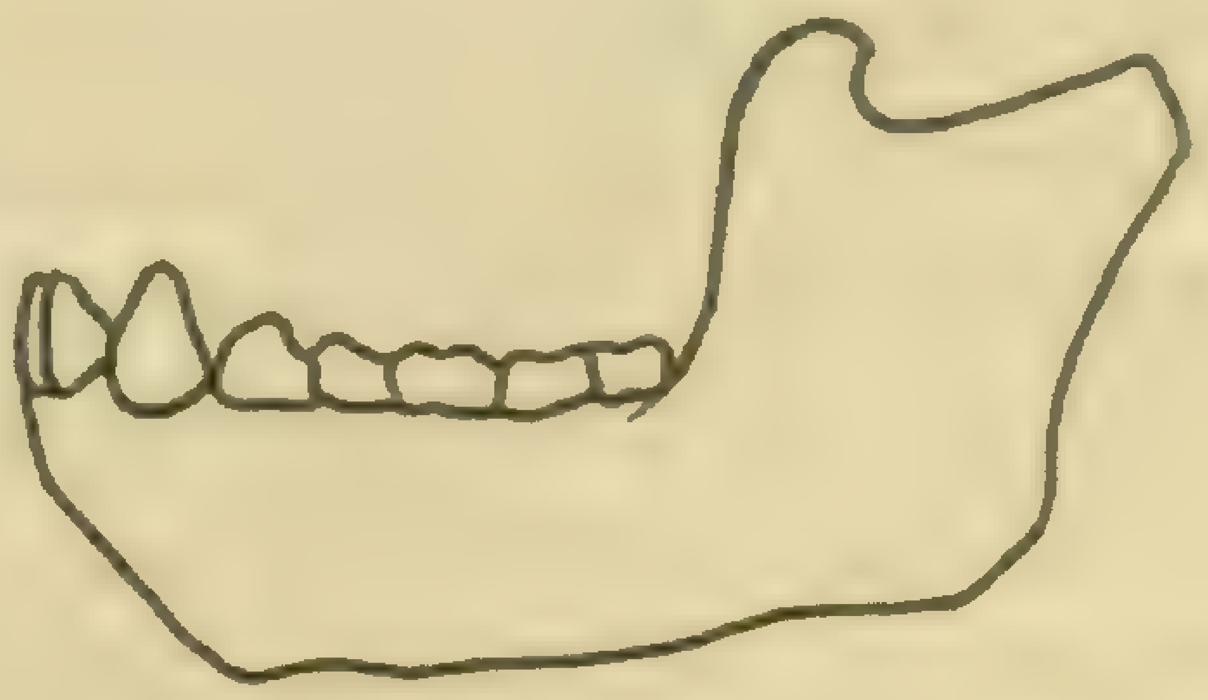

Fig. 2. Lower jaw of an orang, showing the absence of chin.

the man of to-day from the more ancient of his progenitors. The earlier fossil skeletons of man are quite chin-

less. The absence of this bony projection in the face is in fact one characteristic thing in our more or less ape-like forefathers (Figs. 3 and 4). The question is, how the "evolution" of this chin is to be explained.

Dr. Robinson's explanation seems to me to boil down to this: that man is, before all other creatures, a talker. In talking, the genio-glossus muscle is called upon to do the most work. This is a fan-shaped muscle which composes a large part of the under portion of the tongue, and is attached to the inner surface of the jaw just within

FIG. 3. The lower jaw of an ancient ancestor of man; the "*Eoanthropus dawsoni*," an early Pleistocene form from Piltdown, Sussex (sketched from the restoration by Dawson and Woodward).

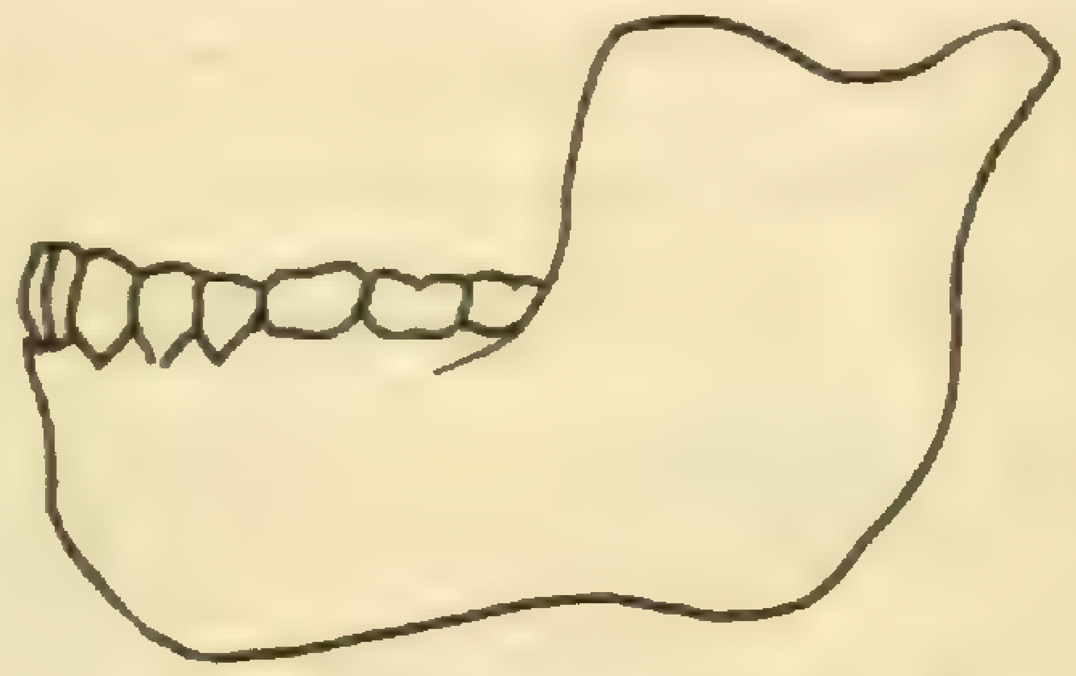

FIG. 4. The lower jaw of *Homo heidelbergensis*, a Pleistocene ancestor of recent man, found at Mauer near Heidelberg.

the chin. It is, according to Robinson, larger, more specialized in structure and more fully fasciculate in man than in the monkeys. The chin, then, says Robinson, is the point of origin for this elaborate muscle, which in a minute of conversation makes several hundred separate movements. The chin has crowded forward in its present conspicuous form as successive generations of men developed more adequate apparatus for speech. In other words, the chin developed because of the use and the consequent development of this one "talking-muscle."

It is only fair to remark that this is an old discussion. Walkhoff, in a series of papers, beginning with a volume edited by Selenka in 1901,[1] put forward the theory now rejuvenated by Robinson. The suggestion was critically reviewed by Fischer in a series of articles.[2] Since then the idea has appeared in a variety of journals.

[1] *Ausz. Biol. Centralbl.*, Volume 22.

[2] Especially *Anat. Anz.*, Volume 23 (1903); Volume 25 (1904).

It is only proper to say, further, that Robinson's various statements about the matter are hardly consistent. He states that the chin is, in origin, merely a buttress for the canine teeth; and he also believes it to be the result of sexual selection. Having accounted for it in these two ways, he throws in his remarks about the genio-glossus muscle for good measure. He closes by spending more discussion on the genial tubercles than on the chin itself. If the first of the statements to which reference has been made is correct, those animals which have large canines ought to be found with the best-developed chins. Quite the opposite is the case. Generally speaking, animals with very large canines, such as the baboons and others, are conspicuous for their very lack of chin. The author also makes certain sensational statements about the lower jaws of the "lower" races, that need full discussion; assuming in one place that uncivilized peoples have phonetically simple languages, an assumption which is startlingly contrary to the facts. His assumption that the genio-glossus muscle is the one prime factor in speech is not borne out by phoneticians, as he himself notes in one place (page 305). Aside from such minor points, all of which demand argument, I should like to point out what seem to my mind to be some of the more important reasons for considering his theory of the origin of the chin imperfect.

In the first place, if man's chin develops from his talking habit, all other animals, without exception, should lack chins altogether. None of them have a language, properly speaking. Robinson himself, to go no further, mentions other animals, notably the elephants, who do possess chins. The latter have it, as the saying is, to spare—much more than a human being has. Robinson points out quite correctly that talking and the chin develop together, as we observe man evolving through various types. This does not necessarily mean, as Robinson seems to assume, that talking produces the chin. On the contrary, the gluteus maximus muscle undergoes tremen-

dous development throughout the same period which brought in highly specialized language. No one has ever suggested, however, because it develops along with highly specialized language, that this muscle is concerned in speech. I should say that the proper method is to see whether there is any general tendency which would pro-

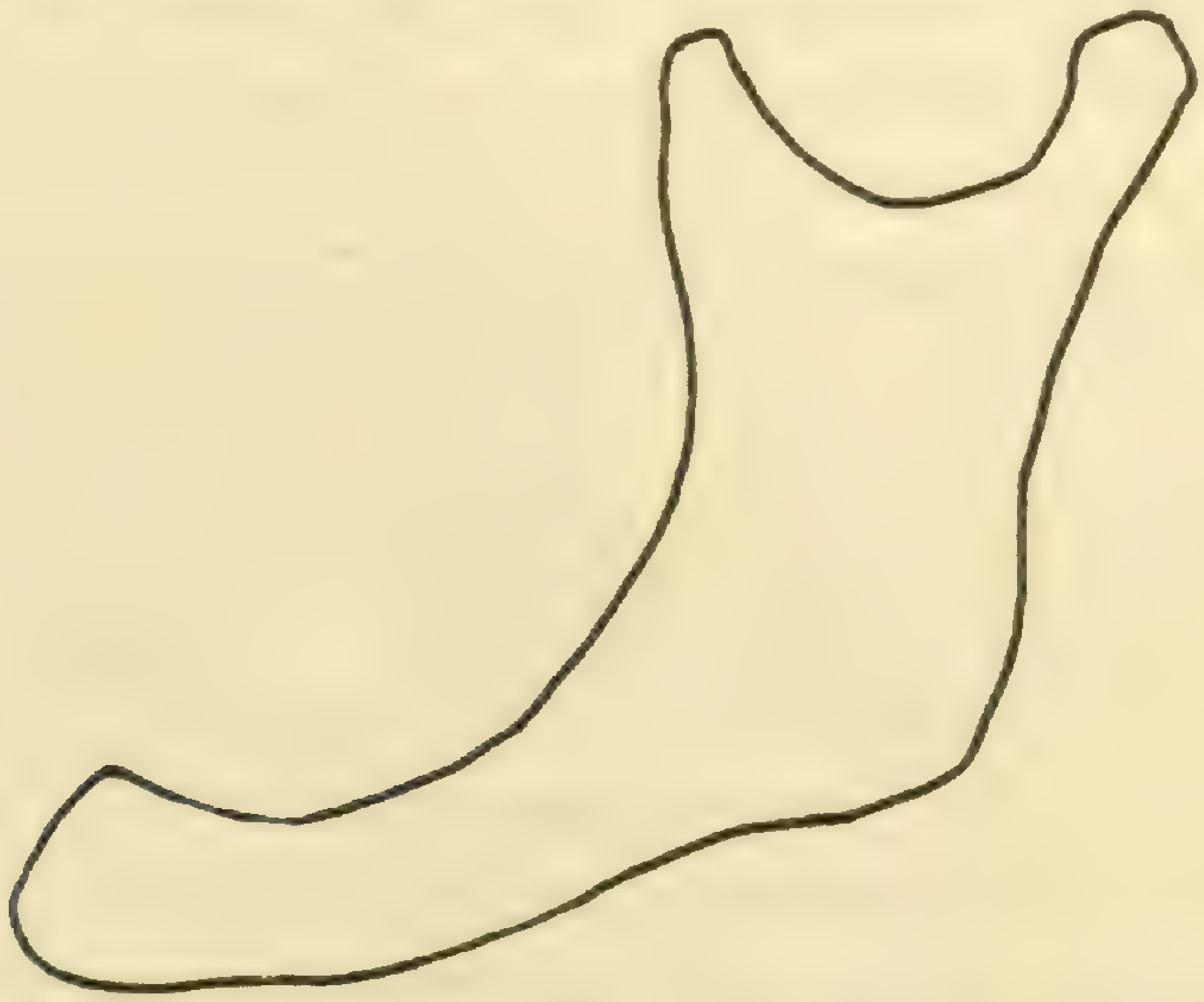

FIG. 5. The lower jaw of an aged person, showing the reabsorption of the alveolar border.

duce chins in the course of evolution, a tendency which would operate in the case of other animals, and also in the case of man and his forerunners. I think there is such a general principle, and a very simple one. I should be inclined to explain the chin, not as a by-product of speech, but as a result of a general reduction in the size of the jaw.

The man-like apes have very heavy chin-less jaws, which, in point of absence of chin, compare with the jaws of the great dogs or cats. Fossil man, too, exhibits, in the more ancient types, enormously large jaws. One general fact, then, in the evolution of modern man, has been a reduction in the size of this part of the body structure. This reduction went along with wider intelligence in the selection of food, and has perhaps been accelerated in man's case by the invention of cooking and other artificial treatment of food-substances. It is, then, a general tendency in the evolution of the human and related types. If we can not explain it, we may at least recognize

it. The next question is: if the jaw is in this way being reduced, should we naturally expect it to be equally reduced in all directions? There are reasons why we might anticipate that it would not.

If we consider especially the horizontal ramus of the jaw, the fact is striking that not all parts of it are equally permanent. The teeth themselves, and the upper border of the ramus, are temporary structures. In old age, the teeth are lost. The upper margin of the jaw itself is, in late life, reabsorbed (Fig. 5), which, with a corresponding loss in the upper jaw, produces the well-known nutcracker appearance of the aged human face. Without attempting to dogmatize, I will go so far as to say that we might confidently expect that the region in the lower jaw which is lightest in structure, and the first to disappear in the individual, would be the part which would naturally respond first to the influence of external environment. Put in another way, the suggestion might be worded thus: We recognize as the important thing in the jaw the *teeth*. Hence, as smaller teeth became more appropriate through change of habit and environment, changes would first appear in these teeth themselves, and in the tissues which immediately support them. In fact, in the fossil "Heidelberg" jaw the teeth have been reduced faster than the jaw itself.[3] Granted that our ape ancestor had a jaw, it

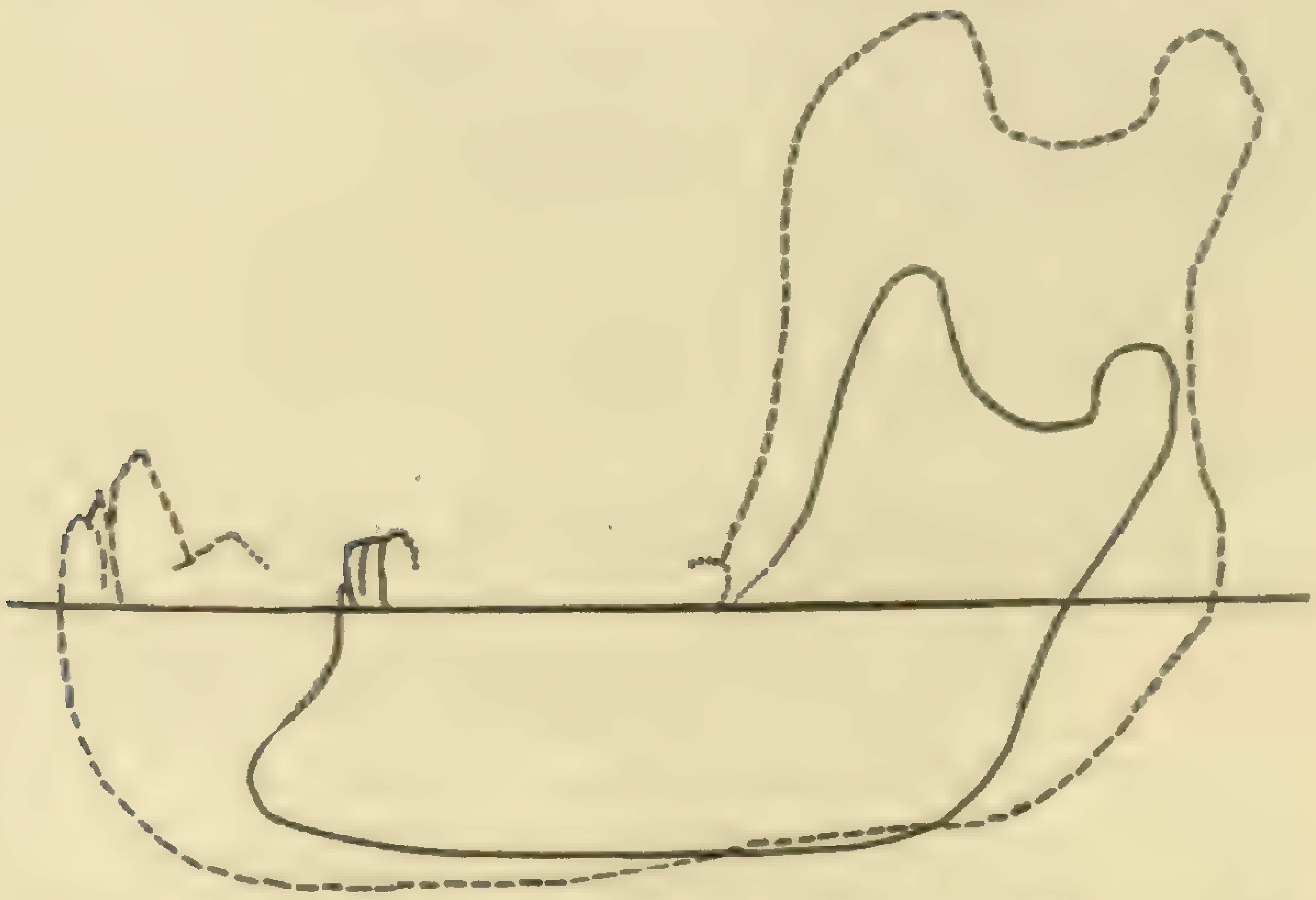

FIG. 6. Lower jaw of the gorilla (broken line), compared with the lower jaw of recent man (adapted from Schoetensack). If the recession in successive gorilla jaws were more rapid in the upper than in the lower border, a prominence would be produced as shown in the human jaw.

is to be expected, it seems to me, that during a general contraction in its size, the superior margin would retract more rapidly than the inferior. I am inclined to think that the chin is the persistent lower margin of our large ancestral jaw. This margin has become retracted more slowly than the upper margin, and therefore juts out into space (Fig. 6).

A difficulty immediately suggests itself. If a *human* chin results from reduction in the size of the jaw, wherever in different species of animals jaws have become reduced, we ought logically to find chins. One striking case can be cited in line with this suggestion. The case of the elephant suggests itself at once, and very clearly. We know that his jaw bones are the result of a remarkable retraction. The process is one of the most picturesque that we know about.[4] We duly find in the geologically recent elephants, and especially in the living species, a tremendous chin (Fig. 7). Whether still other

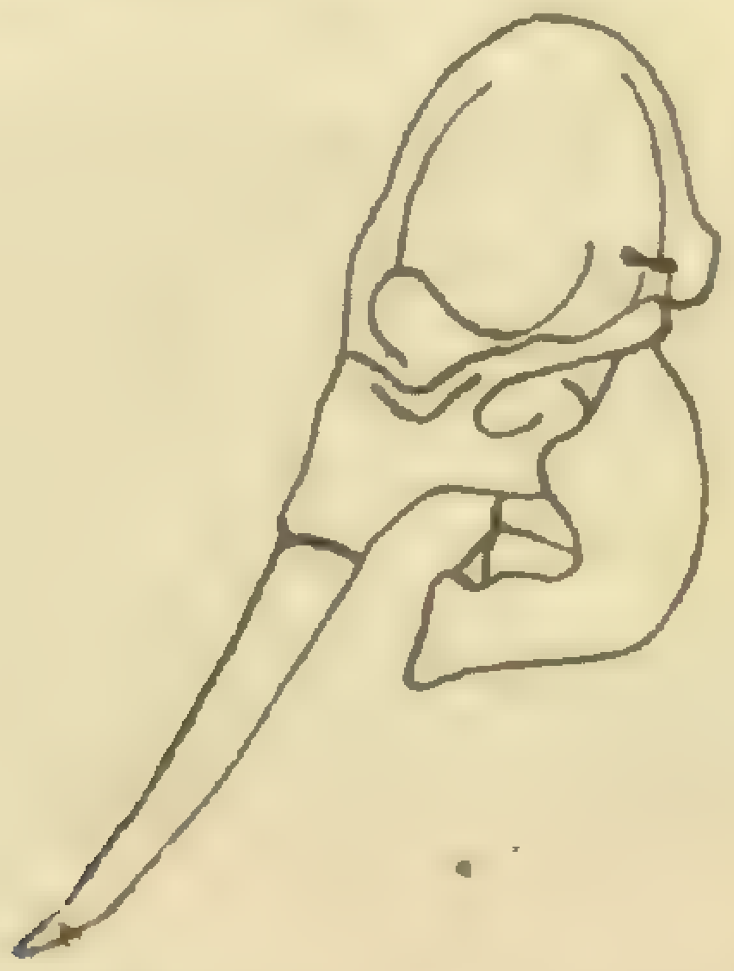

FIG. 7. Face of the Indian elephant (recent), showing the presence of a conspicuous chin which has resulted from reduction in the length of the jaw.

cases of chins resulting from retraction could, or could not, be cited, I do not know. I very strongly suspect, however, that a thorough knowledge of paleontology would put one in position to cite a considerable number, though possibly few cases would be so clear as that of the elephant. I can not resist the feeling that in some such process we have the explanation, not only of human chins, but the chins of other animals as well.

[3] A fact mentioned by MacCurdy in the Smithsonian Report for 1909 (page 570).

[4] Interestingly described by Sir Ray Lankester, "Extinct Animals."

HYBRIDS OF THE GENUS EPILOBIUM

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THAT hybridism and sterility are closely related has been long recognized in a general way, but it is only within the last few years that a systematic and comprehensive investigation, at least of the plant kingdom,

FIG. 1. Flower of *Chamænerion angustifolium*, Southern Ontario.

has been attempted. Professor Jeffrey and his students,[1] working on the flora of eastern North America, have

[1] Jeffrey, E. C., "The Mutation Myth," *Science*, N. S., 39: 488–491, 1914; "Spore Conditions in Hybrids and the Mutation Hypothesis of De Vries," *Bot. Gaz.*, Vol. LVIII, No. 4, Oct., 1914; "Some Fundamental Morphological Objections to the Mutation Theory of De Vries," AMER. NAT., 1915; Holden, R., "Anatomy as a Means of Diagnosis of Spontaneous Plant Hybrids," *Science*, N. S., 38: 932–933, 1913; "Anatomy of a Hybrid Equisetum," *Amer. Jour. of Bot.*, May, 1915.

demonstrated that the infertility of hybrids is due to the abnormal development of the gametic elements, particularly the pollen grains, and have shown that whenever the purity of a species is unquestionable, the spores are uniform, in both size and shape, while, conversely, the spores of hybrids are usually irregular, some appearing normal and others being shrunken and devoid of proto-

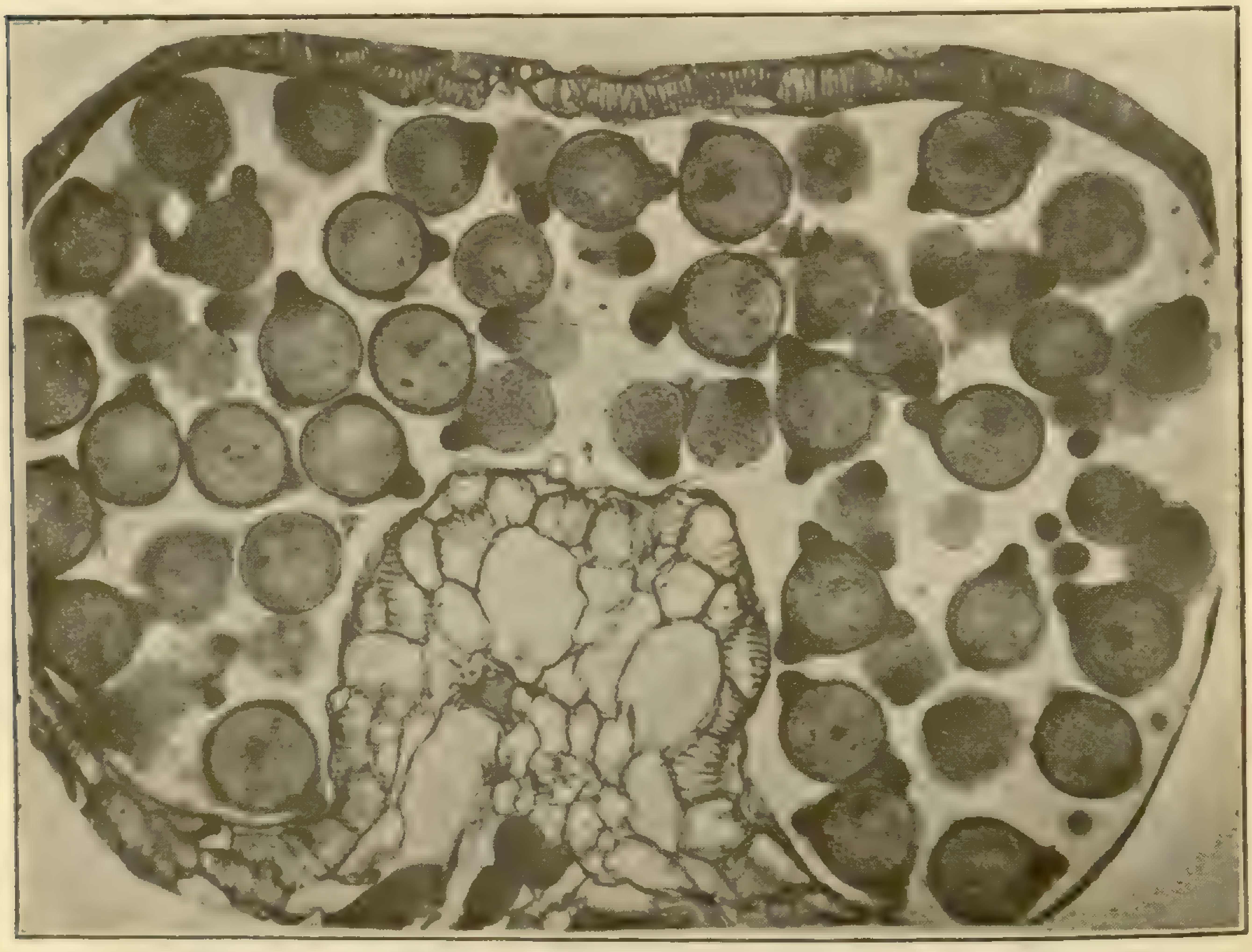

FIG. 2. Half of Anther of *C. angustifolium*, Southern Ontario.

plasm. During the past year the writer has extended these investigations to include a considerable number of English species. Many interesting cases have been encountered, which will be elucidated in detail on another occasion, but the conditions in the genus *Epilobium* are so diagrammatic and typical, that it seems advisable to describe them now.

This genus is divided into two sections, *Chamænerion* and *Epilobium* proper, the chief differences being that in the former the flowers are irregular and the spores not in tetrads, while in the latter the flowers are regular and the spores are persistent as tetrads. Both in eastern

North America and in England, the former section is represented only by *E. angustifolium* (L.), while the latter includes numerous species. Moreover, although the species of the *Epilobium* section are generally recognized to hybridize freely with one another, they do not hybridize with the *Chamænerion* section. Accordingly, one would expect to find only good pollen in the anthers of *E. angustifolium*, and a mixture of good and bad in all the others. Investigation of the North American forms showed that such was indeed the case, and photomicrographs illustrating these conditions were published.[2] When the writer came to examine English specimens, however, a different state of affairs was discovered. Abortive spores were found not only in *E. montanum*, *E. parviflorum* and *E. hirsutum*, as might have been an-

FIG. 3. Flower of *Chamænerion angustifolium* (Hardwick, England). Showing shrivelled anther.

ticipated, but also in *E. angustifolium*. *E. angustifolium* grows wild in only two localities in the vicinity of Cambridge, Hardwick and Gamlingay, but in specimens from

[2] *Loc. cit.*

both these places, as well as in others from the botanical gardens of Cambridge University, the same mixture of good and bad grains was found. These facts seemed to invalidate the conclusion that abortive spores are an invariable sign of hybridism, but, as has so often been the case in scientific matters, evidence which at first seems to discredit a given hypothesis, on further investigation is seen actually to corroborate that same hypothesis.

Reference to systematic works shows that there are two varieties of *E. angustifolium* growing in England, *E.*

FIG. 4. Transverse section of *Chamænerion angustifolium*, showing abortive pollen. (Hardwick, England.)

macrocarpum (Steph.) and *E. brachycarpum* (Leight). There are a number of minor differences in the length of the stolons, shape of the leaves, flowers, etc., but the most definite is the relative length of capsule and pedicel. *E. macrocarpum*[3] grows sparingly but generally from Somerset and Hants. to Orkney, while *E. brachycarpum*, though cultivated commonly all over England, is found much more rarely in the wild condition, being recorded

[3] Boswell, Syme, "English Botany."

from Shropshire, N. Wales, Yorkshire, and even near Edinburgh. Through the kindness of Dr. Wilmott the writer was able to examine the spores of a considerable number of both these varieties from specimens in the herbarium of the British Museum, and in every case the anthers contained a mixture of normal and abortive grains.

We have here, then, a very interesting condition—wherever the two varieties of *E. angustifolium* are present, the spores are partially abortive—indicating the bar sinister; this state of affairs is found in England, and probably in Europe, Asia and western North America, where both varieties are known to coexist. Wherever, on the other hand, as in southeastern North America, there is but one variety, the spores are all normal. *Chamænerion,* therefore, instead of discrediting the value of abortive pollen grains as a test for hybridism, affords another instance of its value. It also suggests another question—how far apart genetically must individuals be before the spores begin to degenerate? Hitherto it has been assumed that only crosses between recognized species bring about that result, but in the case of *Epilobium,* the varietal difference appears sufficient. This, however, opens up the whole question of what is a species, and can not be entered upon here.

SHORTER ARTICLES AND DISCUSSION

CAN SELECTION CAUSE GENETIC CHANGE?

It is almost a pleasure to have occasion for controversy with a fellow worker who shows himself so fair-minded and generous an opponent as does Dr. Pearl in the AMERICAN NATURALIST for February, 1916. He credits my investigations with greater merits than I have claimed or can claim for them. If they possess any superiority, it is not because they have been either better planned or better executed than Dr. Pearl's, but only because the material used was more favorable. In my experiments with rats I have simply undertaken a less difficult task than that undertaken by Dr. Pearl in relation to the fecundity of fowls. Pearl is right in supposing that I have no desire to convey the impression that his work is valueless. No one has greater admiration than I for the masterly way in which he has analyzed the fundamental problems of genetics and the thorough and systematic way in which he has attempted their solution. I regret only that he has courageously attacked so complex a problem before certain simpler and more elementary ones had been solved. I felicitate myself only on having been content with a less ambitious program.

I am pleased to learn too that we are so closely in agreement as regards the observational facts, that in reality it is only concerning the *interpretation* of results that our views seriously differ.

I am quite ready to grant that we are concerned with the same fundamental question, that of the possible quantitative change in a character under selection, that the methods which we have employed are substantially the same and that these methods are open to similar objections, that *random sampling* occurs in the rat experiments as well as in those with fowls, though it is involved in a further degree in the experiments with fowls because of limitations of age and sex. I am quite willing that Pearl should recall the statement "that phenotypic variation of the character fecundity in fowls, markedly transcends, in extent and degree, genotypic variation," and that he should substitute in its stead the statement that it "*may*" so transcend. I am even

more ready to concede the existence of genotypic variation in this character than Pearl has shown himself to be. And I have been reluctant to accept at its face value Pearl's statement that at the conclusion of his fecundity selection experiments he had *more* good winter layers than at the beginning, but none *better*. For in our selection experiments with rats it is very clear that when high-grade individuals grow common, a few individuals of *higher* grade are sure to put in an appearance. Genotypic variation seems to me to be of such wide occurrence that it is difficult to believe that it is ever wholly absent, that absolutely pure lines really exist. I quite agree with Pearl's conclusion that somatic character is not a sure index of genetic constitution and that it was therefore entirely logical and necessary for him to make progeny tests in order to classify his pullets genetically. To establish the point it is not necessary for him, as he observes, "to be fussily nasty" by citing page after page from my Mendelian writings. I had granted the point years before it was raised.

This brings us again to what Pearl considers "the most serious phase of Castle's attack, namely that in which he denies the validity of my conclusions respecting the inheritance of the character fecundity in fowls." Let it be made very clear at the outset *what* is attacked. Not the idea that fecundity is inherited. I think that I am even more ready than Pearl to admit that fecundity is a quantitatively variable character and that its various quantitative conditions are inherited. This is merely to state in another way that *genotypic* as well as *phenotypic* variations in fecundity occur. If they occur, it is possible to isolate them and thus to produce families characterized by them. The conclusion which I "attack" is this, that the observed variations in fecundity depend upon two and *only two* differential factors, both of which are Mendelian, one sex-linked and the other not sex-linked. Several possibilities are conceivable, which this conclusion does not include, as for example that *more* than two genetic factors are concerned in the variation, that one or other or both of the supposed factors are quantitatively variable and so capable of gradual change under selection. I am not advocating or defending any of these possibilities. I am merely attacking the conclusion outlined substantially as I understand Pearl to hold it. There are really several distinct points in this conclusion, some of which seem to be better grounded than others. If I were asked either to accept or to reject it *as a whole* (and Pearl's pub-

lished data leaves no alternative to this) I should reject it, and this decision would not be influenced by the consideration that Morgan, Doncaster, Johannsen and Plate accept it, because it accords with the conception of the pure-line which they have adopted. Authority does not count in science. Majorities do not decide what is true. If they did, Mendelism would have been false in 1868 and true in 1900. If Morgan and Johannsen should next week decide against the pure line idea, as Jennings has already done, what could the rest of us then do except change our minds too, if we base our scientific judgments on authority? Dr. Pearl, I am sure, would be the last to advocate such an idea.

I grant to Pearl the legitimacy of his method in attacking the problem of the inheritance of fecundity and the necessity of establishing arbitrary categories of winter egg production in which his birds are then classified. But I regret what seems to me to be the needless restriction of his published data to the contents of these categories. Pearl points out that I too have made use of arbitrary categories in dealing with the rat statistics, but I would call attention to this difference in our procedure. My categories, + 1, + 2, etc., are indeed arbitrary, but I have not limited the reader's information to their contents. I have published the data in such form that the reader may, if he chooses, form new categories with different inclusiveness, subdividing each category and then subdividing these again down to the lowest limit of observation which can be made with certainty. Pearl has not made it possible for us thus to deal with his data. We may take it or leave it, but we can not change it. We have no means of knowing how many pullets laid 1–10 eggs in their first winter, how many laid 11–20, or 21–30 eggs. In what particular are these "original records" which Pearl withholds "valuable" except as proof of the conclusions which he sees fit to base on them? If he decides, as announced, that the data are not to become public property until he has finished his own study of them, he is well within his rights, but what is the hurry about forcing the *conclusions* upon a waiting public? Would not the public be justified in deferring its decision as to the validity of those conclusions until data as well as conclusions are available?

Pearl seeks to offset his own sin of omission by charging a like offence upon me, maintaining that the scientific public withholds acceptance from my conclusions concerning the rat selection experiments solely because I have never presented my results "in

such form that any other interpretation of the data could by any chance be tested.'' If this statement is true, it is because of my inability to devise any other form in which to present the data. I have presented it in such form that the limits adopted for the categories of variation could be shifted at will and I am ready to be shown how its presentation can be further improved and simplified. Pearl suggests that my omission pertains to the individual pedigree of the rats, in which suggestion he echoes a thought of the Hagedoorns on which I have twice commented elsewhere, showing, I think, that the alleged defect does not exist, for the following reasons:

1. It is impossible for a colony of 33,000 rats to be produced from an original stock of less than a dozen animals, with constant breeding together of those which are alike in appearance and pedigree, and with continuous selection of extremes in two opposite directions, without the production of pedigrees which in the course of each selection experiment interlock generation after generation and finally become in large part identical with each other. This has been repeatedly verified in individual cases, but is incapable of a more generalized statement or of demonstration in generalized form. At least I am unable to devise such demonstration.

2. In a specific case described on pp. 20 and 21 (Castle and Phillips) a selection experiment was started with the hooded F_2 offspring of a single selected hooded and a single wild rat and this experiment was carried through the F_8 generation leading to the production of 804 young from rigidly selected, closely inbred descendants of a single pair. We showed (l. c., p. 21) that the progress of selection within this inbred family follows a remarkably close parallel, generation by generation, to the progress of selection in our plus series as a whole. Here there can be no question of a difference in pedigree among the selected animals. This is eliminated as a possible factor in the result. Can Pearl suggest any other possible factors capable of elimination? If so, I should be pleased to give attention to them.

I humbly beg pardon for having made the all too obvious suggestion that environmental conditions, and in particular size of flock, may affect average flock fecundity. And yet I find that Pearl himself elsewhere lays great stress on this point. My chief offense seems to lie in my failure to realize that he had already taken all possible precautions in this matter, and that he consid-

ered himself in a position to vouch for the uniformity of environmental conditions, not only in eight years of experiments which he had personally superintended, but also in nine previous years of experiments of which he had neither control nor information until they were completed and which were made sometimes in 50, sometimes in 100, and sometimes in 150 bird flocks. Are there not here some elements of uncertainty which at least condone the offense even if they do not excuse the question?

I am prepared to accept without question Pearl's statement that date of hatching can not possibly have had anything to do with the rise in average flock production which has occurred between 1908 and 1915, notwithstanding his own previous statements on the subject and the evidence which Phillips has produced that date of hatching of ducks affects their adult size. I am prepared to accept the view that this rise was due wholly to genetic changes, but I do not believe that Pearl or any one else is in a position to say to what agencies the decline previous to 1908 was due.

And now, with Pearl, I turn with pleasure to the general problem of selection and note that our differences are here rather verbal than real. They lie in that philosophic pitfall of *causation.*

Pearl can not conceive that selection may *cause* or *occasion* or *lead to* genetic change, though he can readily see how populations may change under its influence. Thus selection may increase the proportion of high-grade individuals but it can not, on his view, beyond a limited and fixed point, occasion the production of individuals of increased grade. With these views I squarely take issue, and I shall try to show that his view is a purely *a priori* view, while mine is based on both observation and experiment.

Pearl's reasoning throughout rests on the assumption that the potentiality of a germ cell can not change except by a *causeless* method, "mutation"; that no extraneous influences can change it. Experience teaches directly the contrary, indicating that germ-cells brought together in fertilization mutually *influence each other.* Let us consider for a moment Pearl's illustration. He supposes an organism to exist, A_{38}, which is producing gametes of the uniform value, a_{38}, and can not understand how such gametes uniting with each other can ever produce individuals of a higher value, say A_{39}. No more can I, if we accept his further

hypothesis that there is to be "no mixing of germ-plasms." But what justification have we for that further hypothesis? Experience furnishes none. On the contrary, I have shown in numerous specific cases that when unlike gametes are brought together in a zygote, they mutually influence each other; they partially blend, so that after their separation they are less different from each other than they were before. The pure-line advocates have adopted the procedure of dismissing such explanations as mystical, an easy way to dispose of troublesome ideas. But the stubborn fact remains to be accounted for that partial blending does occur (1) when polydactyl guinea-pigs are crossed with normals (Castle, 1906), (2) when long-haired guinea-pigs are crossed with short-haired ones (Castle and Forbes, 1906) and, (3) when spotted guinea-pigs or rats are crossed with those not spotted (MacCurdy and Castle, 1907). Davenport has furnished numerous instances of the same thing in poultry; indeed he has shown that "imperfection of dominance" and of segregation are the rule rather than the exception in Mendelian crosses in poultry. To assume that "there is no mixing of germ-plasms" is a contrary-to-fact assumption, whatever it may be in formal logic or scientific methodology.

Let us change slightly Pearl's hypothetical case. Let us suppose, as he does, that a gamete a_{38} has united in fertilization with another a_{38} gamete producing a soma, A_{38}. Now what sort of gametes may we expect such an individual to produce? Pearl says, in effect, nothing but a_{38} gametes, unless a genetic miracle occurs, a mutation, incapable of casual explanation. But we should hesitate to characterize as miraculous anything which occurs with regularity, and experience shows that this is what happens quite commonly, if not regularly, in such cases. The A_{38} individual produces gametes a *majority* of which have the value a_{38}, but a few of which have a higher value, a_{39}, and a few a lower value, a_{37}. For the correlation in value between soma and gamete is not absolute. It is in many cases close, but not invariable, as I think Dr. Pearl would admit. If it be granted for the sake of argument that gametic variation occurs, it is obvious that we have grounds for expecting somatic variation in the following generation. For an a_{39} gamete uniting with another gamete like itself may be expected to produce a zygote of value A_{39}. Pearl maintains that such an event is without "causation," is incapable of prediction and control, that all we can do is to

record its occurrence, a view I by no means share. But, it may be asked, *what control* can we exercise over the event? We can prevent or permit it at will. For observation shows that if we permit the individual to mate only with those of inferior value, we shall get no offspring of the highest grade. Thus $a_{38} + a_{36}$ produces commonly only A_{37}, rarely A_{38} and, we might say, never A_{39}. But if we permit the individual to mate with individuals of *equally* high grade (and this is what selection in a particular direction does) experience shows that a majority of the offspring will be of that same grade, but a *few* will be of higher grade. These few make possible further advances. Thus a_{39} makes possible the subsequent attainment of a_{40}. Whether this relationship involves "causation" or not is a question for the logicians and methodologists, of whom I am not one. As to the *fact* our rat experiments leave no doubt. In the light of such facts it seems to me that a view earlier held among biologists, that variability is one of the fundamental properties of organisms, comes nearer to the truth than this modern notion of the pure, unvarying line. This pure-line concept Pearl rightly characterizes as "one of the most useful working tools in the practical breeding of plants and animals that has ever appeared." Why useful? Because it has caused us to pause and take careful inventory of our facts, and to discard as rubbish many loosely held notions. But Pearl reminds us that not all the pure linist's facts are in one basket with Johannsen's beans, nor even in that other vanished basket with Jenning's paramecia. There are, he reminds us, "all the Svalöf oats and wheats to be reckoned with." True and they are mute witnesses to the cumulative effects of selection. For all agree that these pure lines of oats and wheats are the product of continuous self-fertilization. And what more than self-fertilization renders possible generation after generation the union of gamete with its like, the indispensable condition for progressive variation in a particular direction, as I have tried to show?

Intelligent selection only accelerates this natural process of progressive variation, for it singles out the individual which is producing gametes of unusual value and permits the union of such high grade gametes only with gametes of their own sort, so that step after step in a particular direction becomes possible, where unguided self-fertilization would give only halting and uncertain progress. Can we doubt that it is progressive varia-

tion guided by rational selection in a particular direction that has made possible the doubling in size that most of our domesticated animals have undergone since they were taken from the wild state? And does any one seriously think that a *single* selection from wild stock has produced for us the enormous horses of Flanders, or the little ponies of the Shetland Isles, the enormous sheep of the Scotch highlands, or the huge rabbits of Europe, each a monstrosity in comparison with its most probable wild ancestors, and yet producing blends in crosses with them? This blending shows that the change has been one of slow accomplishment and not the result of sudden discontinuous change.

When we compare the color varieties of domestic animals with those of their wild ancestors, as I have been able personally to do in the case of cavies, we are struck by the fact that the domestic varieties are relatively clear and distinct in color, either more intense, more dilute, or of purer color than we can obtain from the wild form by simple recombination of genetic factors. For example it is possible by crosses to obtain from wild cavies the retrogressive varieties, black and yellow. But such synthetic blacks lack the full intensity of blackness found in our best strains of black guinea-pigs, and the synthetic yellows are apt to be either pale or muddy in yellowness, lacking the intensity and brilliancy of our best domestic varieties. It is impossible to escape the impression that our improved domestic varieties are not mere factorial recombinations derived from wild species, but that they have been forced up to a higher standard by repeated selection; that the breeder, for example, has first observed variation in intensity of blackness among his blacks (doubtless obtained originally from a retrogressive sport) and that by repeatedly selecting the blackest available individuals he has increased the blackness of the race. Thus it is no accident that the meat and milk and wool producing capacity of our domestic animals far exceeds that of any wild ancestral species. The standard in each case has been raised and it has not been raised by a single lucky accident (the mutation view), but by a series of slow advances each impossible until a previous advance had been made. I am aware indeed that Pearl at one time maintained an opposite view, holding (if I remember correctly) that our best strains of poultry are no better layers than *some* strains of jungle fowl. But I do not believe that this view can be successfully defended. I am certain that such an idea is quite preposterous in

the case of most characters for which our domestic animals are valued and as regards which their *improvement* has been attempted by selection. In such cases there has been a *series* of slight advances, and everything indicates that the *order* of the advances is significant and necessary, that the higher stages can be attained only by passing through the lower ones. If this is so, we need not quibble about "*causation,*" but we may assure ourselves that if we wish to attain a distant goal, the first thing to do is to make for intermediate points.

I regard it as a hopeful sign that Pearl can see no reason why genetic changes may not be small in amount in some cases, even though large in others. This I hope is only a first step toward the complete abandonment of that "real, genuine pure-line body of doctrine" which he still holds dear.

W. E. CASTLE

BUSSEY INSTITUTION,
FOREST HILLS, MASS.,
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GENERAL BIOLOGY OF THE PROTOZOAN LIFE CYCLE[1]

GARY N. CALKINS, PH.D.

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FOR five decades after the time of Ehrenberg, the peculiar conception of a protozoan as a miniature replica of a metazoan, held by this gifted observer, influenced the study of Protozoa. This influence gradually wore off and, so far as morphology is concerned, ended with the careful observations of Stein, Claparède and Lachmann, Engelmann, Bütschli and Hertwig, who showed that various structures of the protozoan body are not beating hearts, brains, ovaries and stomachs, but are simple differentiations of the single-celled organisms.

A more lasting influence of Ehrenberg's teaching, seen even to-day, is the habit of regarding a single protozoon as the complete expression of a species equivalent to an individual worm, mollusc or mammal. The individual metazoon dies, while the protozoon does not die but grows to full size and divides into two or more—facts which led Weismann to his conclusions regarding mortality in Metazoa and immortality in Protozoa.

We owe to Maupas the credit for dissipating this last reminiscence of Ehrenberg's teaching, and for showing that the single cell is not the final representative of a protozoon species. We are accustomed to the idea that many

[1] Opening address Subsection E, Protozoology, Section VIII 2nd Pan-American Congress.

individuals of a polymorphic coelenterate are present in potential in the fertilized egg of the coelenterate, but we are less accustomed to the idea that polymorphic individuals are present in potential, in the fertilized cell of a protozoon. Research in recent years has shown that successive generations of Protozoa may be more or less progressively differentiated, so that a cell picked out at one phase of the life cycle is quite a different type of individual from one picked out at another phase. Which, for example, would be the "type" individual of the dimorphic Foraminifera? Which would be the type in the reproducing flagellated and ameboid stages of *Nägleria punctata?* of different phases in the life history of *Centropyxis, Arcella,* or *Difflugia?* or of intestinal and blood-dwelling stages of *Plasmodium?* The morphological differences here indicate that the protozoan life history involves differentiation analogous to that of a polymorphic metazoon, and justify the comparison of the whole life cycle with the development and differentiation of a metazoon, especially that of a metagenetic type such as coelenterate or trematode.

The importance of the whole life cycle, first demonstrated by Maupas, was fully recognized by Schaudinn and applied by him to the study of parasitic forms. The monographs resulting from this study, especially those on *Coccidium schubergi, Plasmodium vivax* and on rhizopods, are classics in the literature of Protozoa, and models which later students have followed.

Through Schaudinn's work, and by later researches, the sequence of events in different parasitic types has been made out with painstaking care until to-day, we know the general history of the majority of injurious human protozoan parasites, the modes of transmission from host to host, the types of intermediate hosts and what happens in them. In short, we know enough to furnish an adequate basis for public and private prophylaxis which, in the hands of sanitary commissioners and public health officers, has put an end to epidemics of yel-

low fever, malaria and dysentery; has rehabilitated vast tracts of land in Italy; saved millions of dollars in South Africa and in our southern states, and has made the Panama Canal possible.

Such are the first, and practically the most important, results of our knowledge concerning protozoan life cycles; quite enough, indeed, to justify the science of Protozoology. Important as these results are, we are not at all satisfied; we know too little about the conditions of development; too little about the nature of the vital processes of the organisms themselves and their variations in structure and function under differing conditions, ignorance which must be cleared away before much further practical advance can be made. Further advance will be less spectacular and must be based upon the biological study of the organisms as units of protoplasmic substance, and this will rest upon working hypotheses supported by experiment. It is along such theoretical lines that I wish to direct your attention for a few minutes, to develop a conception of the life cycle as a whole, and to offer a theoretical interpretation of the different phases of vitality and of structural variations.

Let us consider for a moment, a single *Ameba* or a malaria germ, not as a cause of disease, but as a unit mass of protoplasm which, like a free-living *Paramecium* or *Didinium*, performs all of the fundamental vital activities common to living things, namely nutrition, excretion, irritability and reproduction. The chemical composition of these unit masses, so far as I know, has never been made out, but there is no reason to doubt that it agrees with that of other living substances, since the accompanying properties of protoplasm—metabolism, growth and reproduction—are obviously performed, and probably in the same way. In such unit masses of protoplasm we assume that processes of hydrolysis, synthesis, oxidation and reduction, are constantly going on as in other protoplasms, and not in any haphazard way, but always orderly and under regulative control of the organism as a whole.

The appearance of *Ameba* shows that the protoplasm is made up of alveoli and inter-alveolar substances of different density, representing colloidal and crystalloidal substances in a general mixture which Ostwald describes as an emulsoid. Between these different substances constant chemical activities are in progress, and the orderliness which distinguishes these processes in the protoplasm of the living organism from similar processes which go on in the same protoplasm when crushed, are possibly due, as Mathews states, to the physical barriers of cellular and nuclear membranes, alveoli, and the colloidal centers of activity. The speed with which such processes take place in living protoplasm, which, in itself, distinguishes living processes from chemical processes in lifeless substances, is due to specific enzymes or catalyzers which are manufactured as a result of chemical activities in living protoplasm. These bring about and control each successive step in the long chain of chemical actions involved in destructive metabolism, the action in each event being conditioned by the nature of the protoplasmic substratum. In this chain of destructive processes different substances may be formed which undergo no further oxidation or other chemical change, but are stored up in the protoplasm until disposed of by excretion, these products, leading to changes in the protoplasmic substratum, *i. e.,* to protoplasmic chemical differentiation, may or may not be accompanied by visible structural differentiations. Such products of destructive metabolism, in the form, usually, of nucleo-proteins or their derivatives, may act as poisons to other organisms, as melanin does to the host in malaria, or as the proteolytic ferments of *Entameba histolytica* do in dysentery; or they may play some important part in the vital activities of the organism itself, as in phosphorescence of *Noctiluca* and the dinoflagellates, or more generally, in regeneration and reproduction.

Let me illustrate this latter point by some experiments made on *Uronychia transfuga,* a ciliated protozoon. This organism has rather a complicated structure with nine

giant cirri at the posterior end (Fig. 1). Under laboratory conditions it divides once a day approximately, or, more exactly, once in twenty-six hours. The first indication of division is the precocious formation of the giant cirri in a central region of the body which we have called the "division zone." The experiments were undertaken for the purpose of studying the relative power of regeneration of the single cell at different ages between divisions, it having first been determined that the cell regenerates readily after being cut. Cells were cut with a scalpel at different periods subsequent to division; some during the end stages of division; some 15 minutes after division; some one hour after; others 2, 4, 8, 12, 16 and 20 hours after, and some were cut just prior to the next division period, *i. e.*, 24 to 25 hours after division. In all cases of record, the cells were so cut that one portion contained the micronucleus and part of the macronucleus, the other portion containing only a part of the macronucleus. The former, or, as I shall call it, the nucleated portion, invariably regenerated after some hours, forming a perfect cell, the latter, without a micronucleus which I shall call the enucleated portion, behaved differently as regards regeneration, according to the age of the cell when cut. In all cases this portion lived from three to five days after the operation. If the recently divided cell were cut at any period up to 16 hours after division the result was the same; no regeneration occurred, the fragment merely rounded out, swimming about by its adoral membranelles (Fig. 2, 3). If the cells were cut when from 18 to 24 hours old, regeneration occurred not only in the nucleated portion, but in the *enucleated fragment as well*, the percentage of regeneration increasing with the increased age of the cells when cut, until at the age of 24-25 hours the enucleated fragments regenerated perfectly in 100 per cent. of cases (Fig. 4, 5, 6, 7).

These results indicate a gradual chemical differentiation of the protoplasm as a result, probably, of destructive and constructive metabolic processes. The giant

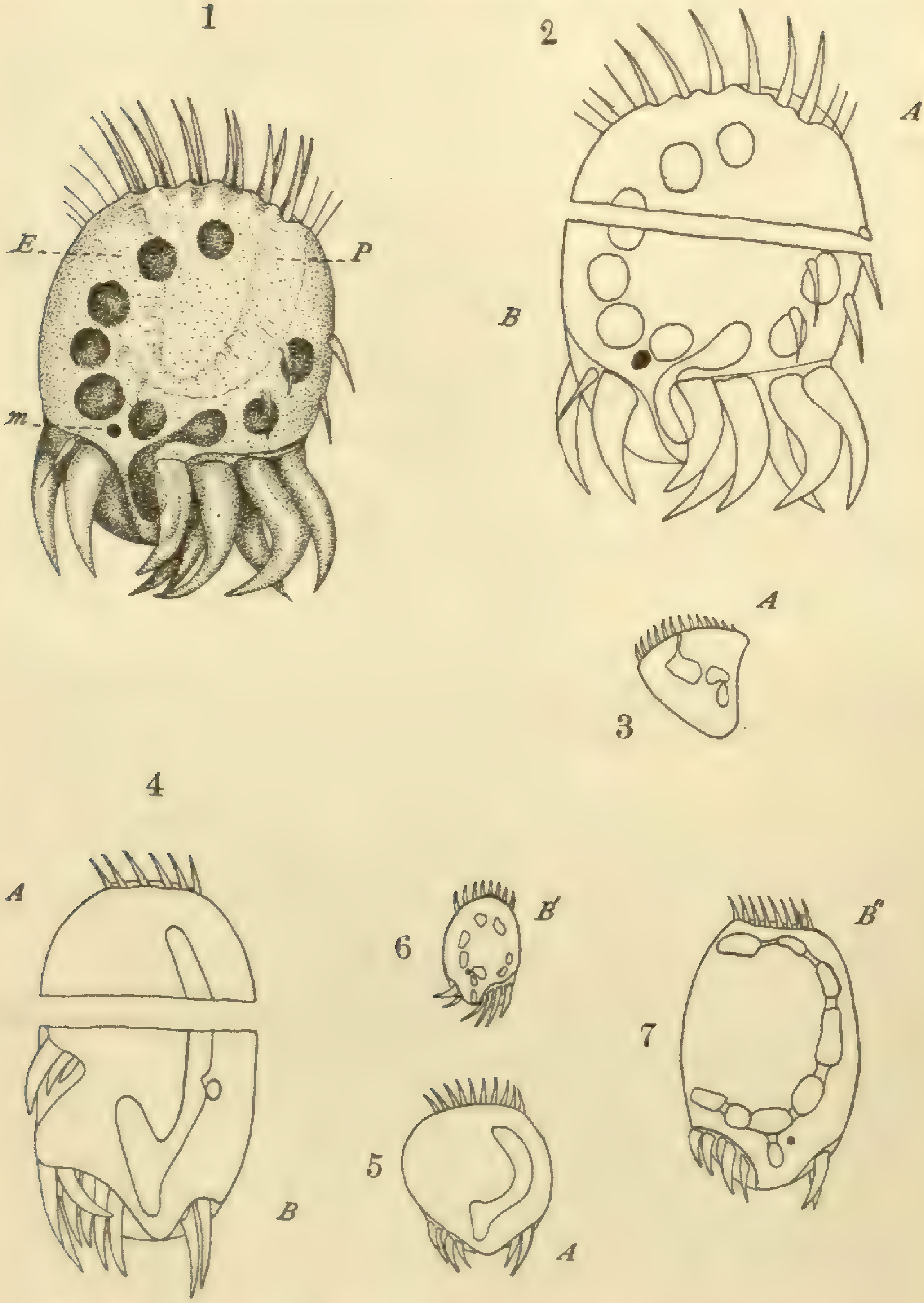

FIG. 1. Normal adult individual of *Uronychia transfuga* with macronucleus in the form of large chromatin spherules; micronucleus (*m*); endoral membrane (*E*); pre-oral membrane (*P*); and large posterior cirri.

FIGS. 2 AND 3. Individual 12 hours old cut as shown in 2. Part *A* had no micronucleus and after 72 hours appeared as shown in 3 *A*. Part *B* regenerated perfectly in 24 hours.

FIGS. 4, 5, 6 AND 7. Individual cut at age of 25 hours as shown in 4. *A* regenerated perfectly, except for absence of micronucleus, in 24 hours (5 *A*); *B* divided through the original division plane (indicated in 4), within a few hours forming a minute but perfect individual (6B'), and a normal full-size individual (7 *B''*).

cirri which are regenerated are the visible expression of inherited structures characteristic of the species. Since the enucleated fragment from a cell cut when young does not regenerate while the nucleated fragment does, we must conclude that one essential factor at least, necessary for the production of these inherited structures, lies in the micronucleus.

The giant cirri, furthermore, are visible differentiations which are precociously formed at division. This must mean that the inherited factors find their expression at this period, and it follows from the successful formation of giant cirri in enucleate fragments from old cells, that whatever may be the direct causative agent or agents in the process they must be generally distributed throughout the protoplasm at this time. We have no direct evidence as to what these agents may be; possibly there is only one and that of the nature of a specific enzyme, or perhaps some chemical body analogous to hormones formed as a result of mutual interaction of nucleus and cytoplasm when the latter has reached a certain stage of chemical differentiation through normal activities. Or it is possible that such chemical bodies are present at all times and are activated only when the protoplasmic substratum reaches some particular stage of development. Thus it is possible that, with continued metabolism, the acidity of the protoplasm gradually increases until a concentration is reached in which specific enzymes, not able to act before, are now activated.

However theoretical the interpretation of the phenomenon may be, the periodic and temporary power of regeneration is an observed fact indicating a difference in the protoplasmic make-up at different age periods, a difference which may be satisfactorily expressed by the phrase cumulative chemical differentiation.

Another observed fact is that the regenerative power is exhausted with cell division, for young enucleated fragments do not regenerate. This indicates a reduction of the differentiated adult protoplasm to the condition of young cells; or, at least, the protoplasm is restored to a

state where the causes underlying regeneration are inactive. This may be due to the exhaustion of specific substances which take part in the reaction of regeneration, or it may be due to the chemical and physical changes accompanying cell division.

We are led through these experiments, to further speculations concerning the nature of cell division. Chemical differentiation of the protoplasm continues even after the stage is reached when regeneration is possible. This is shown by the fact that formation of the cirri in *Uronychia* precedes the process of division in normal cells, and by the additional fact that regeneration of cirri occurs while cell division does not occur in enucleate fragments cut from old cells. I would interpret cell division as due to cytolytic action set up by enzymes or other chemical bodies produced as a result of interaction of nucleus and cell body differentiated chemically by age. Cytolysis may then occur more or less extensively throughout the entire protoplasmic mass, but it is most active in the division zone of the organism which is more highly differentiated than other regions (see Calkins, 1911, and Peebles, 1912). The membrane of the cell turns in at this cytolyzed division zone and the constriction results in cell division.

As a consequence of the activities accompanying cell division the protoplasmic substratum is reduced from the differentiated adult condition to the condition characteristic of young cells, and the processes of growth and chemical differentiation, division and de-differentiation, recur in more or less rhythmical succession.

Viewing the life cycle as a whole, there are two phases which must be taken into account. These are, first, the encystment phase, and second, the sexual or conjugation phase, both widespread and almost universal in protozoan life histories. Let us first consider the encystment phase.

Encystment occurs ordinarily when the conditions in the surrounding medium are adverse, such as desiccation, lack of food, etc., such encysted forms emerging from the cyst when suitable conditions are restored. In some cases also, encystment occurs during the digestion of food. In

addition to these casual encystments there is another form of encystment which involves more deeply-lying activities of the protoplasm. In *Didinium nasutum* I have found that encystment occurs at periodic intervals which cannot in any way be connected with adverse conditions of the environment or with feeding, but must be interpreted as a normal phenomenon due to internal conditions of the organisms. Encystment at such times persists for from 5 to 8 days and during this period no amount of coaxing will bring the organisms out. During such encystment the macronucleus fragments into hundreds of small chromatin particles which are ultimately absorbed in the cytoplasm; the micronuclei divide, and products of their division give rise to a new macronucleus and new micronuclei. When the process is completed and the organisms emerge from their cysts they possess from five to seven times the vitality, as measured by the division rate, of the same race prior to encystment. Fermor was the first in 1913 to describe similar happenings during the encystment of *Stylonychia;* in this case, dissolution of the old macronucleus and absorption of the fragments, fusion of the two micronuclei and formation of new macronuclei and micronuclei from the fusion nucleus, were described.

It is well known that *Paramecium* does not encyst. Nevertheless Woodruff and Erdmann (1914) have shown that phenomena similar to those occurring during encystment in *Stylonychia* and *Didinium*, and which they refer to under the general term "endomixis," recur at periodic intervals (about once a month) in the case of *Paramecium aurelia*. Here also the old macronucleus fragments and the fragments are absorbed in the cytoplasm, while a new macronucleus and micronuclei are formed from the division products of the old micronuclei.

The interpretation of this set of phenomena in the life history of protozoa is a perplexing problem. There is not a doubt that vitality, as measured by the division rate, is restored. Likewise there is little reasonable doubt that a complete chemical and physical reorganization of the protoplasm takes place. The renewal of vitality was shown

both in Woodruff's culture and in my *Didinium* culture, and one general problem is stated in the query: how long can such periods of reorganization continue? Woodruff believes that they may keep on indefinitely, but in my experiments with *Didinium* the race apparently lost its power to encyst and ultimately died out after six months' culture without encystment. So too, in my culture of *Paramecium caudatum* (1902) where similar reorganization occurred at least twice, the race ultimately lost the power to reorganize and died out. I may have had unfavorable forms to start with and so lost both races at early dates. It is interesting in this connection, however, to note that Whitney, working with the rotifer *Hydatina,* a metazoon, carried a race through nearly 200 generations by parthenogenesis when the individuals lost their power to reproduce in this way, and many of his lines died, while others produced sexual individuals.

The general biological effect of this process of reorganization is a new chemical combination with a new potential of metabolic activity, and a new lease of life. Not only are the nuclei restored to activity, but the cytoplasm is likewise completely reorganized by the distribution through it of relatively large quantities of nucleo-proteins, giving rise to successive derivatives (through hydrolysis, oxidation, reduction, etc.), all increasing the metabolic processes and releasing more chemical energy expressed by activity of movement and feeding, and leading to more rapid assimilation and growth, all indicated by an increased division rate. In short, the protoplasm is rejuvenated.

The second phase in the life history to be considered, viz., the sexual phase, involves still more deeply-reaching protoplasmic activities. The protoplasm of the individual cells at this period has a different physical, and presumably chemical, make-up than during ordinary vegetative periods. In free-living forms, such as the ciliates, the outer protoplasm becomes sticky or glutinous so that two cells on touching, fuse together. In this condition which I have called the "miscible state" conjugation is possible, and the physical condition may be so extreme that groups

of cells get stuck together. I have witnessed the fusion of nine *Paramecium caudatum* cells in a single amorphous mass.

In other forms, notably the parasitic protozoa, protoplasmic changes at this stage follow two lines of differentiation. Some cells store up metabolic products in the form of reserves of nutriment and develop into female gametocytes or macrogametes. Others develop into more active male gametocytes and microgametes. In both of these differentiated types if union or fertilization is prevented, the cells die a natural death.

The effects of conjugation or fertilization are almost the same as those following asexual reorganization through encystment. In ciliates cytolysis of the old macronucleus takes place and its substances are absorbed, that is, undergo chemical changes in the cytoplasm. The majority of the maturation nuclei, both in free-living and in parasitic forms, meet the same fate, while a new nuclear apparatus results from the products of the fertilization nucleus or synkaryon. The cytoplasm is renewed in a chemical sense and metabolic activities recommence with renewed vigor; a new race is started. The sole difference from encystment is that reorganization occurs after or during amphimixis and a new hereditary complex is formed in the nucleus, while even this, in endogamous conjugation at least, can not be very different from the condition after asexual reorganization. It is obvious that, if conjugation is the equivalent of fertilization in metazoa, asexual reorganization or endomixis is the equivalent of parthenogenesis.

What is the significance of these two important phases in the life cycle and how can they be interpreted in terms of metabolic activities? As we have seen, there is reason to believe that the cell protoplasm becomes progressively differentiated in a chemical sense between division periods, until just prior to division processes take place which do not occur at earlier periods. With division this differentiated condition is reduced, possibly through cytolysis, until a more labile protoplasm results. Now it is not at

all improbable that such reducing processes are more or less incomplete, so that the protoplasmic substratum in the second generation is different from that of the first. We have evidence of this in the foraminifera where differences in the protoplasmic structure and in shell structure characterize the second generation. Further evidence is seen in the rhizopods, where increasing quantities of chromidia, and in some cases differences in shell structure, are morphological indications of differentiation.

Furthermore, it is not improbable that such differences are cumulative from generation to generation, just as chemical differentiation is cumulative with inter-divisional age, until a protoplasmic substratum is evolved in which processes not possible before can now take place. We have shown that *Paramecium* at the conjugation phase has a different physical make-up than at other times, the cortical plasm becomes mucilaginous and fusion results on contact, while physiological differences are manifested by the invariably decreasing division rate during and after this period when conjugation is possible. Here the protoplasmic substratum is differentiated, and processes occur which are not possible at other times. So, too, in *Didinium*, *Stylonychia*, etc., with successive generations a protoplasmic substratum is gradually evolved (possibly hastened by adverse conditions) in which the peripheral zone of protoplasm undergoes cytolysis and forms an impervious membrane—the cyst membrane—analogous to the fertilization membranes of metazoan eggs. Further cytolytic changes, involving hydrolysis, reduction and other chemical activities, are set up in the cell body, especially in the cell nuclei which divide or fragment. As a result of these activities, which are more profound than those accompanying cell division, the protoplasm is again restored to a labile condition, vitality is renewed and a de-differentiated protoplasm begins a new cycle of metabolic and reproductive phases.

The phenomena of conjugation may be interpreted in a similar way as due to processes possible only in a substratum produced by cumulative protoplasmic differentia-

tion. A visible expression of such differentiation is seen again in the chromidia formation of *Sarcodina* and in the dimorphic gametocytes of foraminifera and Sporozoa. The reorganization phenomena are quite as complicated and as far reaching as after encystment, and the end result is the same, a de-differentiated protoplasm and a new individual with a high potential of vitality. If fertilization is prevented the differentiated macro- and microgametes die as do metazoan eggs and spermatozoa, and a similar result follows the continued culture of free-living ciliates in which conjugation, or its equivalent, asexual endomixis, is prevented.

In all life histories we find more or less regular cycles of vegetative and sexual phases, complicated by more or less active asexual and sexual reproduction. In parasitic forms it is possible, I may say probable, that reorganization and renewal of vitality take place during encysted stages as Schaudinn, Wenyon and others have held for the genus *Entameba;* or, as in *Paramecium,* they may take place without encystment in types like *Plasmodium* as described by Schaudinn. The processes of autogamy, so-called, described for different types of *Entameba,* may be interpreted as asexual endomixis, and the conflicting views as to the significance of nuclear structures in *Entameba coli, E. histolytica, E. tetragena* and *E. minuta,* may all be reconciled when this possibility of asexual reorganization is applied to the various parasitic rhizopods.

With *Plasmodium,* the principle of asexual reorganization and renewal of vitality, or parthenogenesis, has long been called upon to explain malaria relapse. The process, as described by Schaudinn, is too familiar to need repetition here. Despite the objections which have been raised in recent years against this interpretation, it must be admitted that no *à priori* difficulty stands in its way. It is evident from experiments that the protoplasm of an old race is more stabile than that of a young race, possibly due to accumulation of products of metabolism in the former, either for a useful purpose, as in the storage of yolk material in a female cell, or for some harmful purpose, as in

Paramecium caudatum during depression. In either case if a labile protoplasm can be restored resulting in chemical activities which ultimately bring about dissolution of these formed products, then renewed vitality is the outcome. Asexual reorganization effects this result, but the same result was produced artificially by the use of salts in my experiments with *Paramecium caudatum* during conditions of depression, and in cases where the cell body was visibly loaded with products which it could not automatically dispose of. The splendid results which Bass has obtained in cultivating *Plasmodium* in vitro and in the presence of sugar, indicate the possibility of malaria organisms while in a stabile condition being similarly changed into a labile condition by changes in the blood content of the host. Changes thus set up might well be the equivalent of asexual reorganization or parthenogenesis, or the equivalent of fertilization in restoring vitality.

In this sketch of the protozoan life cycle I have endeavored to give a comprehensive though somewhat speculative account of the different phases of vitality which may apply equally well to any type of Protozoa. Cell division, reorganizing encystment or its equivalent, and conjugation, are all regarded as phenomena of the same general character but differing in degree, the effect in each step being the restoration of the protoplasm to a condition more or less free from cumulative metabolic differentiations.

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THE EVOLUTION OF THE CELL. III

BY THE LATE PROFESSOR E. A. MINCHIN, F.R.S.

In the phase of evolution that I have termed the pseudomoneral or cytodal phase, in which the organism was a droplet of periplasm containing scattered biococci or chromidiosomes, metabolism would result in an increase in the size of the cytode-body as a whole, accompanied by multiplication of the chromidiosomes. Individualization of the cytodes would tend to the acquisition of a specific size, that is to say, to a limitation of the growth, with the result that when certain maximum dimensions were attained the whole cytode would divide into two or more smaller masses amongst which the chromidiosomes would be partitioned.

In the next stage of evolution, the protocyte with a definite nucleus, it is highly probable that at each division of the cell-body, whether into two or more parts, the primitive method of division of the nucleus was that which I have termed elsewhere "chromidial fragmentation";[26] that is to say, the nucleus broke up and became resolved into a clump of chromidiosomes, which separated into daughter-clumps from which the daughter-nuclei were reconstituted. Instances of nuclear divisions by chromidial fragmentation are of common occurrence among the Protozoa and represent probably the most primitive and direct mode of nuclear division.

It is clear, however, that if the chromatin-grains are to be credited with specific individuality and qualitative differences amongst themselves, this method of nuclear division presents grave imperfections and disadvantages, since even the quantitative partition of the chromatin is inexact, while the qualitative partition is entirely fortuitious. Chromidiosomes having certain specific properties might all become accumulated in one daughter-cell,

[26] *Op. cit.*, p. 101.

and those having opposite properties in the other, so that the two daughter-cells would then differ entirely in their properties.

I can but refer briefly here in passing to the interesting theory put forward by Bütschli, to the effect that sexual phenomena owe their first origin to differences between cellular organisms resulting from the imperfections of the primitive methods of cell-division. If we assume, for instance, as so many have done, that one of the earliest qualitative differences between different chromatin-granules was that while some influenced more especially the trophic activities of the cell, others were concerned specially with kinetic functions; then it might easily happen, after nuclear division by chromidial fragmentation, that all, or the majority of, the kinetic elements pass into one of the two daughter-cells, while its twin-sister obtains an undue preponderance of trophic chromatin. As a consequence, some cells would show strong kinetic but feeble trophic energies and others the opposite condition, and in either case the viability of the cells would be considerably impaired, perhaps inhibited. If it be further assumed that cells of opposite tendencies, kinetic and trophic, attract one another, it is easy to see that the union and fusion of two such cells, the one unduly kinetic (male) in character, the other with a corresponding trophic (female) bias, would restore equilibrium and produce a normal cell with kinetic and trophic functions equally balanced. On this view, sexual union, at its first appearance, was a natural remedy for the disadvantages arising from imperfect methods of nuclear division.

It is not surprising, therefore, to find that the process of nuclear division undergoes a progressive elaboration of mechanism which has the result of ensuring that the twin sister-granules of chromatin produced by division of a single granule shall be distributed between the two daughter-cells, so that for every chromatin-grain obtained by one daughter-cell an exact counterpart is obtained by the other; in other words, of ensuring an exact qualita-

tive, as well as quantitative, partition of the chromatin-particles. In its perfect form this type of nuclear division is known as karyokinesis or mitosis, and all stages in its progressive development are to be found in the Protozoa.

In the evolution of nuclear division by karyokinesis two distinct processes are being developed and perfected in a parallel manner, but more or less independently; first, the method of the partition and distribution of the chromatin-grains between the two daughter-nuclei; secondly, the mechanism whereby the actual division of the nucleus and the separation of the two daughter-nuclei are effected in the cell-division. I have dealt elsewhere[27] with the evolution of the mechanism of karyokinesis as exemplified by the numerous and varied types of the process found amongst the Protozoa, and I need not discuss the matter further here, but the behavior of the chromatin-grains may be dealt with briefly. The main feature in the process of the exact quantitative and qualitative distribution of the daughter-chromatin between the daughter-nuclei is the aggregation of the chromatin-grains or chromioles into definite, highly individualized structures known as chromosomes. In the most perfected forms of the process of chromosome-formation the chromioles become united into a linear series termed by Vejdovský a chromoneme, which is supported upon a non-chromatinic basis or axis. According to Vejdovský, the supporting substance consists of linin; R. Hertwig, however, in his well-known studies on *Actinosphærium*[28] considers that the supporting and cementing substance of the chromosome is plastin derived from the substance of the nucleoli. However that may be, the essential feature of the chromosome is the cementing together of the chromioles to form the chromoneme, a thread of chromatin which may be disposed in various ways on the supporting axis, sometimes being wound spirally round it (Vejdovský).

[27] *Op. cit.*, pp. 105–120.

[28] *Abhandl. bayer. Akad.* (II. Cl.), XIX, 1898.

The actual division of the chromatin takes place by the longitudinal splitting of the chromoneme, in other words, by simultaneous division into two of each of the chromioles of which the thread is composed. In this way every chromiole which was contained in the original chromoneme is represented by a daughter-chromiole in each of the two daughter-chromonemes. It follows that the familiar process of the splitting of the chromosomes in karyokinesis is a mechanism which brings about in the most simple, sure and direct manner an exact quantitative and qualitative partition of the chromatin-grains between the two daughter-nuclei. In the sequel each daughter-nucleus is built up, according to Vejdovský, entirely and solely from one of the two daughter-clumps of chromosomes, and each chromosome is resolved again into its constituent chromioles, giving rise in some cases to a definite portion of the nucleus, a karyomere, from which again, at the next nuclear division, the chromosome is reconstituted by the chromioles falling into line in an orderly manner.

The chromatin-cycle of a cell in which the process of division by karyokinesis takes place in its most perfectly developed form, may, therefore, be conceived as follows: The nucleus in its resting state contains a definite number of companies or brigades of chromatinic units (chromioles), each brigade spread over a certain extent of the nuclear framework forming a karyomere. As a preparation to division each separate brigade of chromioles falls into line as the chromoneme, forming with its supporting substance the chromosome; there are formed, therefore, just so many chromosomes as there were karyomeres in the nucleus. In this disciplined and orderly array each chromiole undergoes its division into two daughter-chromioles, so that each file or chromoneme of chromioles splits into two files. At the reconstitution of the daughter-nuclei each daughter-chromosome gives rise to a karyomere again, the chromioles falling out of the ranks and disposing themselves in an apparently irregular manner on the

newly-built framework of the daughter-nucleus to constitute their own particular karyomere. Thus karyokinesis differs only from the most primitive method of division by chromidial fragmentation in that what was originally a haphazard method of distribution has become a disciplined and orderly manœuvre, performed with the precision of the parade-ground, but in a space far less than that of a nutshell.

In the nuclear division of Protozoa, without going into excessive detail, it may be stated broadly that all stages are to be found of the gradual evolution of the tactical problem which constitutes karyokinesis. The chromosomes in the more primitive types of nuclear division are usually very numerous, small, irregular in number and variable in size; the splitting of the chromosomes is often irregular and not always definitely longitudinal; and distinct karyomeres have not so far been recognized in the nuclei of Protozoa. In many cases only a part, if any, of the chromatin falls in to form the chromosomes, and a greater or less amount of it remains in the karyosome, which divides directly into two. The various types of nuclear division in Protozoa have been classified as promitosis, mesomitosis and metamitosis, for detailed accounts of which those interested must refer to the textbooks and original descriptions.

I have dealt briefly with the problem of the evolution of karyokinesis because the process of nuclear division is, in my opinion, of enormous importance in the general evolution of living organisms. I have expressed elsewhere[29] the opinion that the very existence of multicellular organisms composed of definite tissues is impossible until the process of karyokinesis has been established and perfected. For tissue-formation it is essential that all the cells which build up any given tissue should be similar, practically to the point of identity, in their qualities; and if it is the chromatin-elements of the cell which determine its qualities and behavior, then the exact qualitative

[29] *Op. cit.*, p. 120.

division of the chromatin, as effected in karyokinesis is indispensable as a preliminary to the production of identically-similar daughter-cells by division of a parent-cell. Hence it becomes intelligible why, amongst Metazoa, we find the occurrence of nuclear division by karyokinesis in its most perfect form to be the rule, and "direct" division of the nucleus to be the rare exception, while, on the other hand, in the Protista, and especially in the Protozoa, we find every possible stage in the gradual evolution of the exact partition of the chromatin in the process of nuclear division, from chromidial fragmentation or the most typical amitosis up to processes of karyokinesis as perfect as those of the Metazoa.

There now remains only one point of general interest in the evolution of the cell to which brief reference must be made, namely, the divergence of animal and vegetable cells. Not being a botanist, I desire to approach this question with all caution; but as a protozoologist it seems to me clearly indicated that the typical green plant-cell took origin amongst the Flagellata, in that some members of this group of Protozoa acquired the peculiar chromatophores which enabled them to abandon the holozoic or animal mode of life in exchange for a vegetative mode of nutrition by means of chlorophyll-corpuscles. It is well known that many of these creatures combine the possession of chlorophyll with an open, functional mouth and digestive vacuoles, and can live either in the manner of plants or of animals indifferently or as determined by circumstances. It would be interesting to know exactly what these chromatophores, at their first appearance, represent; whether they are true cell-organs, or whether, as some authorities have suggested, they originated as symbiotic intruding organisms, primitively independent. I do not feel competent to discuss this problem. I would only remark here, that if the green plant-cell first arose amongst the Flagellata, then the distinction between plant and animal (that is, green plant and animal) is not so fundamental a divergence in the series of living beings

as is popularly supposed, but is one which did not come into being until the evolution of organisms had reached a relatively advanced stage, that, namely, of the true nucleated cell.

I have confined myself in this address to the evolution of the cell as this organism is seen in its typical form in the bodies of the multicellular organisms, starting from the simplest conceivable type of living being, so far as present knowledge enables us to conceive it. But there is not the slightest reason to suppose that the evolution of the Protista took place only in the direction of the typical cell of the cytologist. Besides the main current leading up to the typical cell there were certainly other currents tending in other directions and leading to types of structure very unlike the cells composing the bodies of multicellular organisms. It is impossible that I should do more here than indicate some of the divergent lines of evolution, and I will confine myself to those seen in the Protozoa.

Taking as the starting-point and simplest condition in the Protozoa a simple cell or protocyte, in which the body consists of a small mass of cytoplasm containing a nucleus, with or without chromidia in addition, an early specialization of this must have been what I may term the plasmodial condition, typical of Rhizopods in which the cytoplasm increased enormously to form relatively large masses. The nucleus meanwhile either remains single and grows very large or, more usually, a great number of nuclei of moderate size are formed. From this large plasmodial type is to be derived the foraminiferal type, characterized by the creeping habit of life, and probably also the radiolarian type, specialized for the floating pelagic habit. Both foraminiferal and radiolarian types are characterized by an excessive development and elaboration of skeletal structures, and the geological record proves that these two types of organisms attained to a high degree of specialization and diversity

of form and structure at a very early period.[30] The Mycetozoa exemplify another development of the creeping plasmodial type adapted to a semi-terrestrial mode of life.

In the Mastigophora the body generally remains small, while developing organs of locomotion and food-capture in the form of the characteristic flagella. In this class there is a strong tendency to colony-formation brought about by incomplete separation of sister-individuals produced in the ordinary process of reproduction by binary fission. The so-called colonies (they would better be termed families) show a most significant tendency to individualization, often accompanied by physiological and morphological specialization of the component flagellate individuals.

As an offshoot, probably, from ancestors of the Mastigophoran type arose the Infusoria, the Ciliata and their allies, representing by far the most highly organized unicellular type of living being. No cell in the bodies of the Metazoa attains to such a complication of structure as that exhibited by many Ciliates. In the Metazoa the individual cells may be highly specialized for some particular function of life; but a Ciliate is a complete and independent organism and is specialized for each and all of the vital functions performed by the Metazoan body as a whole. From the physiological standpoint a Ciliate (or any other Protist) is equivalent and analogous to a complete Metazoon, say a man, but I can not for a moment agree with Dobell[31] that the body of a Ciliate is homologous with that of a Metazoon—not at least if the word homologous be used in its usual biological sense of homogenetic as opposed to homoplastic. Dobell appears to me to negative his own conclusion when he maintains that the body of a Ciliate is "non-cellular" while admitting that the Metazoon is multicellular; how then can they be said to be homologous? Only if the term homologous be

[30] For Foraminifera see especially Heron-Allen, *Phil. Trans.* (B), Vol. 206 (1915), p. 229.

[31] *Journal of Genetics*, IV (1914), p. 136.

used in a sense quite different from its ordinary significance.[32]

In addition to the highly developed structural differentiation of the body the Infusoria exhibit the extreme of specialization of the nuclear apparatus in that they possess, as a rule, two distinct kinds of nuclei, micronuclei and macronuclei, composed respectively of generative and trophic chromatin, as already pointed out. This feature is, however, but the culminating point in a process of functional specialization of the chromatin which can be observed in many Protozoa of other classes, and which, moreover, is not found invariably in its complete form in all Ciliata.

In this address I have set forth my conceptions of the nature of the simplest forms of life and of the course taken by the earliest stages of evolution, striving all through to treat the problem from a strictly objective standpoint, and avoiding as far as possible the purely speculative and metaphysical questions which beset like pitfalls the path of those who attack the problem of life and vitalism. I have, therefore, refrained as far as possible from discussing such indefinable abstractions as "living substance" or "life," phrases to which no clear meaning can be attached.

How far my personal ideas may correspond to objective truth I could not, of course, pretend to judge. It may be that the mental pictures which I have attempted to draw are to be assigned, on the most charitable interpretation, to the realm of poetry, as defined by the greatest of poets, rather than of science.

> The lunatic, the lover and the poet
> Are of imagination all compact;
>
> . . . .
>
> And as imagination bodies forth
> The forms of things unknown, the poet's pen
> Turns them to shapes and gives to airy nothings
> A local habitation and a name.

If I might be permitted to attempt an impartial criticism of my own scheme, I think it might be claimed that

[32] See Appendix A.

the various forms and types of organisms in my evolutionary series, namely, the simple cell or protocyte, the cytode or pseudomoneral stage, the micrococcus, even the biococcus, are founded on concrete evidence and can be regarded as types actually existent in the present or past. On the other hand the *rôle* assigned by me to each type in the pageant of evolution is naturally open to dispute. For example, I agree with those who derive the Bacteria as primitive, truly non-cellular organisms, directly from the biococcus through an ancestral form, and not at all with those who would regard the Bacteria as degenerate or highly-specialized cells. But the crux of my scheme is the homology postulated between the biococcus and the chromatinic particle—chromidiosome or chromiole—of true cells. In support of this view, of which I am not the originator, I have set forth the reasons which have convinced me that the extraordinary powers and activities exhibited by the chromatin in ordinary cells are such as can only be explained on the hypothesis that the ultimate chromatinic units are to be regarded as independent living beings, as much so as the cells composing the bodies of multicellular organisms; and, so far as I am concerned, I must leave the matter to the judgment of my fellow-biologists.

I may point out in conclusion that general discussions of this kind may be useful in other ways than as attempts to discover truth or as a striving towards a verity which is indefinable and perhaps unattainable. Even if my scheme of evolution be but a midsummer-night's fantasy, I claim for it that it coordinates a number of isolated and scattered phenomena into an orderly, and, I think, intelligible sequence, and exhibits them in a relationship which at least enables the mind to obtain a perspective and comprehensive view of them. Rival theories will be more, or less, useful than mine, according as they succeed in correlating more, or fewer, of the accumulated data of experience. If in this address I succeed in arousing interest and reflection, and in stimulating inquiry and controversy, it will have fulfilled its purpose.

Appendix A.—The Cell-Theory

The most recent attack on the Cell-theory, as it is understood by the majority of modern biologists, has been made by Mr. Dobell, who, if I understand him rightly, refuses to admit any homology between the individual Protistan organism and a single cell of the many that build up the body of a Metazoon. On the contrary, he insists that the Protist is to be regarded as homologous with the Metazoan individual as a whole. On these grounds he objects to Protista being termed "unicellular" and insists that the term "non-cellular" should be applied to them.

As regards the cellular nature of the Protista, it is one of my aims in this address to show that amongst the Protista all stages of the evolution of the cell are to be found, from primitive forms in which the body can not be termed a cell without depriving the term "cell" of all definable meaning, up to forms of complex structure in which all the characteristic features of a true cell are fully developed. Thus in the Protozoa we find the protoplasmic body differentiated into nucleus and cytoplasm; the nucleus in many cases with a structure comparable in every detail to that of the nucleus of an ordinary body-cell in the Metazoa; reproduction taking place by division of the body after a karyokinetic nuclear division often quite as complicated as that seen in the cells of the Metazoa and entirely similar both in method and in detail; and in the sexual process of differentiation of the gametes on lines precisely similar to those universal in Metazoa, often just as pronounced, and preceded also in a great many cases by phenomena of chromatin-reduction comparable in principle, and even sometimes in detail, with the reduction-process occurring in Metazoa. I really feel at a loss to conceive what further criteria of homology between a Protozoon and a Metazoan cell could be demanded by even the most captious critic. On the ground of these and many other similarities in structure and behavior between the entire organism in the Protozoa and the individual cell, whether tissue-cell or germ-cell, in the Metazoa, the case

seems to me overwhelmingly convincing for regarding them as truly—that is to say, genetically—homologous.

Looking at the matter from another point of view, namely, from the standpoint of the Metazoa, it is true that in the groups of most complicated and highly organized structure the cells often develop secondary connections or fusions due to incomplete division, to such an extent that in parts of the body the individuality of the primitively distinct cells may be indicated only by the nuclei (as may occur also in Protozoa, for example, in associated gregarines); but in all Metazoa certain of the cells retain permanently their complete independence and freedom of movement and action. In the Metazoa possessing the simplest and most primitive types or organization, such as sponges and cœlenterates, the cells composing the body show far greater independence of action, and in the course of ontogeny entire groups of cells may alter their relative positions in the body as the result of migrations performed by individual cells; while it is now well known that if the adult sponge or hydroid be broken up completely into its constituent cells, those cells can come together again and build up, by their own individual activity, the regenerated body of the organism. For these reasons it seems to me impossible to regard the body-cells of the Metazoa otherwise than as individual organisms complete in themselves, primitively as independent as the individual Protozoon, and in every way comparable to it.

From the considerations summarized very briefly in the two foregoing paragraphs and capable of much greater amplification and elaboration, the view generally held that the entire organism of a Protozoon is truly homologous with a single body-cell of a Metazoon seems to me quite unassailable, and to have gained in force greatly from recent investigations upon both Protozoa and Metazoa. On the other hand, any Protist, as an organism physiologically complete in itself, is clearly analogous to the entire individual in the Metazoa—a comparison, however, which leaves the question of genetic homology quite untouched.

As regards the application of the term unicellular or non-cellular to the Protozoa, it is evident that if the evolution of living beings had never proceeded beyond the stage of the Protista, and if no multicellular organisms had ever been evolved, the term cell could then never have been invented by an intelligent being studying other living beings, supposing for an instant the possibility of such intelligence existing apart from a mammalian brain. So long as the Protozoa are studied entirely by themselves, without reference to any other forms of life, they may be termed non-cellular in the sense that they are not composed of cells. It is only when they are compared with multicellular organisms that the term unicellular becomes applicable on the ground of the homology already discussed between the Protozoon and the body-cell of the Metazoon.

THE MECHANISM OF CROSSING-OVER

II

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IV. The Manner of Occurrence of Crossing-over

A. Interference

As soon as it seemed probable that the factors were linked in line, and that the crossing-over was the actual method of interchange, it became of interest to discover and to analyze the precise mode of incidence of the interchange. The questions suggested themselves, for example, what was the total frequency of crossing-over, did any factors separate more often than they remained together, how often did crossing-over occur at two points simultaneously, and was there any tendency, in such cases, for the two points of crossing-over to be a definite distance apart, or in definite positions, etc. For answers to these questions might throw light on the mechanism of crossing-over, what cytological phenomena it was connected with, and what stage in synapsis it occurred at.

With these points in view the author calculated the linkage relations that would result on several possible schemes of interchange. The simplest possibility was that the chromosomes always twisted in loops of fixed length, though not of fixed position, and always underwent breakage, with recombination of homologous strands (*i. e.*, "crossed-over" in the technical sense), at each place that the strands crossed one another. In such a case there would always be a definite distance between one point of crossing-over and another; moreover, all factors which were separated by a distance great enough for double crossing-over to occur between them, *i. e.*, by the length of at least one loop, must always have either double or single (or multiple) crossing-over between them. Sturtevant's data, however, showed that this was

not true, and accordingly it had to be concluded that the length of the loop was variable, or that "crossing-over" did not always occur where the strands crossed.

Another possibility was that crossings-over were quite independent of one another, having an entirely random or chance distribution in the chromosome, with reference to each other. This would mean that when crossing-over occurred at one point, another crossing-over would be just as likely to occur coincidently at any other given point—whether this be very near or far away—as when no crossing-over took place at the first point. But this latter scheme would not be that expected on the method of crossing-over proposed by Jannsens and followed by Morgan, for in the stages when Jannsens supposed crossing-over to occur the chromosomes are rather loosely twisted, so that loops of very small length do not occur as often as longer ones (thus, very near one point of crossing-over the strands seldom cross back again). I therefore determined the mathematical relations which would exist between crossing-over frequencies, if crossings-over had a chance distribution with reference to one another, in order to compare these figures with those obtained by experiment. On the assumption that separation between A and B has no influence on separation between B and C, if crossing-over occurs between A and B in 10 per cent. of cases and between B and C in 20 per cent. of cases, then, among those ten cases in a hundred where crossing-over between A and B occurs, 20 per cent. (*i. e.*, 2 cases) would be cross-overs between B and C as well; in other words, the per cent. of double cross-overs would be equal to the product of per cents. AB and BC (formula 1). The easiest way to determine the correctness of the assumption in any given case, therefore, is to compare the observed per cent. of double cross-overs with per cent. AB $\times$ per cent. BC.

Another relation besides was found to hold between the theoretical linkage values, dependent upon the relation in formula 1. For it is easily seen that the number of *separations* between A and C must always be equal

to the sum of the number of crossings-over between A and B, and between B and C, minus all those crossings-over contained in the cases where coincidence occurred, and in which A and C, therefore, failed to separate,—*i. e.*, minus twice the number of cases of double crossing-over. Hence, if formula 1 is correct, then it must also be true that per cent. AC = per cent. AB + per cent. BC — 2 (per cent. AB × per cent. BC) (formula 2). This formula was originally expressed not only in the above terms, where the "per cent. of separations" (*i. e.*, ratio of separations to the total number of cases) is used as the index of separation frequency, but also in terms of the so-called "gametic ratio"—the ratio of cases of non-separation to those of separation—for this was the way of indicating degree of linkage then used by all investigators of the subject. The latter index gives much more complicated formulas, however, and so it was pointed out at the same time that per cent. of separations would afford a much more useful measure of linkage.

Later, Trow also worked out and published the same formula (no. 2)—in terms of the "gametic ratio"—and it is generally known as "Trow's special hypothesis" (17). But on the reduplication hypothesis held by Trow, and by the other English geneticists who do not accept the chromosome explanation, the formula would be supposed to result, not from the fact that crossing-over between A and B was independent of that between B and C, but from the fact that "reduplications" AB and BC were independent, not being disturbed by any "primary reduplication" AC. Adherents of the reduplication hypothesis have been much concerned as to whether or not their results confirmed the assumptions made in Trow's formula, and have in one or two instances calculated that they did. Let us examine for a moment the requisites for proving such a conclusion. As above shown, the whole matter turns on the frequency of coincidence of separations AB and BC (*i. e.*, on the frequency of "double crossing-over") and the question can be settled by determining directly the amount of this coincidence.

If the per cent. of double cross-overs = per cent. AB × per cent. BC (formula 1), then the assumption that separation frequencies AB and BC are independent is correct. As offspring from a back-cross all show what factors they received from the hybrid parent, a back-cross involving the three factors A, B, and C at the same time will answer the question at once, for all the cases of coincident separation (double cross-overs) that occur can be counted. But where the hybrids, instead of being back-crossed, are inbred—a practice followed by adherents of the reduplication hypothesis—then it is impossible to tell which F_2 individuals come from gametes of the classes which we may term double cross-overs, unless one of these classes is the triple recessive, and then the only double crossovers which can be known as such are those very rare individuals that happen to result from the union of two double crossover gametes. The British workers have, therefore, not been able to find the proportion of double cross-overs directly, to compare this with formula 1, but have tried to determine the frequency of coincidence indirectly, by using the method followed in formula 2. That is, they determined the relations existing between frequencies AC, AB, and BC, as calculated from their F_2 counts, for, as above shown, the greater the frequency of double crossing-over, the more will AC be cut down in proportion to AB and BC. And it seemed evident that, if the relation of AC to AB and BC was just that given by Trow's formula (2), then coincidence of separations must have the frequency demanded on the assumption that separations (or "reduplications") AB and BC occur independently of one another. As a matter of fact, however, this method offers no answer to the question, unless almost impossibly large F_2 counts are obtained, for otherwise *the independent random fluctuations of these three values in this kind of count are so great that any deviation in AC due to excess or deficiency of double crossing-over would be quite lost to sight.*

The question was, however, immediately and definitively answered in *Drosophila*, before Trow's paper ap-

peared, by examination of Sturtevant's extensive back-crosses, especially of those involving three pairs of factors at once. As the results did not conform to the formula, it was not published, but as Trow has since raised this question publicly and the adherents of the reduplication hypothesis are still discussing it, it may not be out of place to have given an analysis of it here, and to recall the fact that it had already been tried and rejected. Besides, as will appear below, a discussion of the relations which would exist if crossings-over were independent of one another is a necessary preliminary for a treatment of the relations which do exist between linkage values.

The results showed that double crossing-over does not, as a rule, occur as frequently as would be expected if, as the above formulæ assumed, it were purely a matter of chance whether or not two cross-overs happen coincidently. In a sense, then, the occurrence of one crossing-over interferes with the coincident occurrence of another crossing-over in the same pair of chromosomes, and I have accordingly termed this phenomenon "*interference.*" The amount of interference is determined by comparing the actual per cent. of double cross-overs with the per cent. expected if crossings-over were independent, *i. e.*, if they had a purely chance distribution with reference to each other. Now, the per cent. which would occur on the latter expectation has already been given by formula 1 as per cent. AB × per cent. BC. If, then, the observed per cent. of double cross-overs were divided by per cent. AB × per cent. BC, we would obtain a fraction showing what proportion of the coincidences which would have happened on pure chance really took place. This ratio of observed double cross-overs to the chance expectation appears to me to furnish the most useful measure of interference. The ratio is itself best expressed in per cent., and it may be called the relative coincidence, or simply "coincidence." If the "coincidence" is low, this means that there has been much interference, for most of the double cross-overs expected on chance were prevented from appearing; conversely, if coincidence is high, the

interference must have been very weak. Some illustrations may make the meaning of this index clearer. If, for example, coincidence is 0 per cent. no double crossing-over is occurring; the interference between one crossing-over and another is then complete. If coincidence is 45 per cent., this figure does not mean that 45 per cent. of the individuals are double crossovers, but that 45 per cent. of the number of double crossovers which would be expected as a result of pure chance (whatever that number may have been) actually appeared, 55 per cent. having been "interfered with," or somehow prevented from occurring. If coincidence is 100 per cent., there has been no interference, for the same number of double cross-overs appeared as expected on the ground that the two crossings-over did not interfere with each other's occurrence. 110 per cent. would mean that if one crossing-over occurred, the other was 10 per cent. *more* likely to occur than in cases of random distributions of crossings-over. This would be "negative interference," for as coincidence increases interference decreases.

On Janssens's theory that crossing-over takes place in the strepsinema stage, when the chromosomes are twisted in loose loops, crossing-over would very seldom take place at two points very near together, for this would require a tight twisting of the chromosomes. Accordingly, on this theory interference was to be expected; furthermore it would be expected that interference was very great between crossings-over that were in neighboring regions; but between crossings-over further apart there should be little or no interference. The results were according to this expectation; they indicated strongly that the interference was very great for crossings-over short distances apart, but progressively diminished as the distances considered became greater. The conclusion drawn was that crossing-over took place as postulated on Janssens's theory, when the strands were loosely twisted in strepsinema, although the twisting and crossing-over did not take place in the stereotyped manner suggested as a first possibility, in the earlier part of this section. For there

was evidence that the distances between the two points of crossing-over in double cross-overs were variable; but this again corresponded with the fact that the chromosomes of *Batracoseps* and other forms, as seen under the microscope, did not always twist in loops of the same length. Furthermore, if it be supposed that in most maturing eggs of the fly the homologous chromosomes twist tightly enough to cross at least once or twice, as is certainly the case in *Batracoseps* and many other forms, it must be concluded that at not every point of crossing does actual "crossing-over" (recombination of strands) take place, for it was found that nearly half of the factor-groups emerged without having undergone any crossing-over at all. And this, in turn, corresponded with the observations of Janssens and others, which showed that at some at least of the points of crossing of homologous chromosomes, the latter merely untwisted again without having undergone the "chiasmatype" process. Here, then, was a theory of crossing-over that seemed complete, so far as connecting the genetic facts with the cytological observations was concerned.

B. Possible Mechanisms of Crossing-Over

There is one very unsatisfying point, however, in this original scheme of crossing-over. That is, it postulates that crossing-over occurs at a comparatively late stage in synapsis, when the strands have become very much shorter and thicker than the long delicate threads which first came into contact with their homologues (see Fig. 6). Now, in crossing-over the chromosomes must come into contact, and break, at *precisely* homologous points, otherwise factors would be lost or gained by them when crossing-over occurs. But presumably the factors are set very close together in the line, judging by the fact that mutations in new "loci" (positions in the chromosomes) are still as numerous as ever, and that, if the whole chromosome is packed with factors as close together as, judging by their linkage relations, they seem to be at certain places in it, it must contain at the very least 200 factors. It is

difficult to conceive how this cleavage of ultramicroscopic nicety can take place properly at a stage when the chromosomes are so coarse and short. The observations of Vejdovsky and others, taken in connection with the genetic results from *Drosophila*, render it practically certain that the factors are really disposed in an extremely fine,

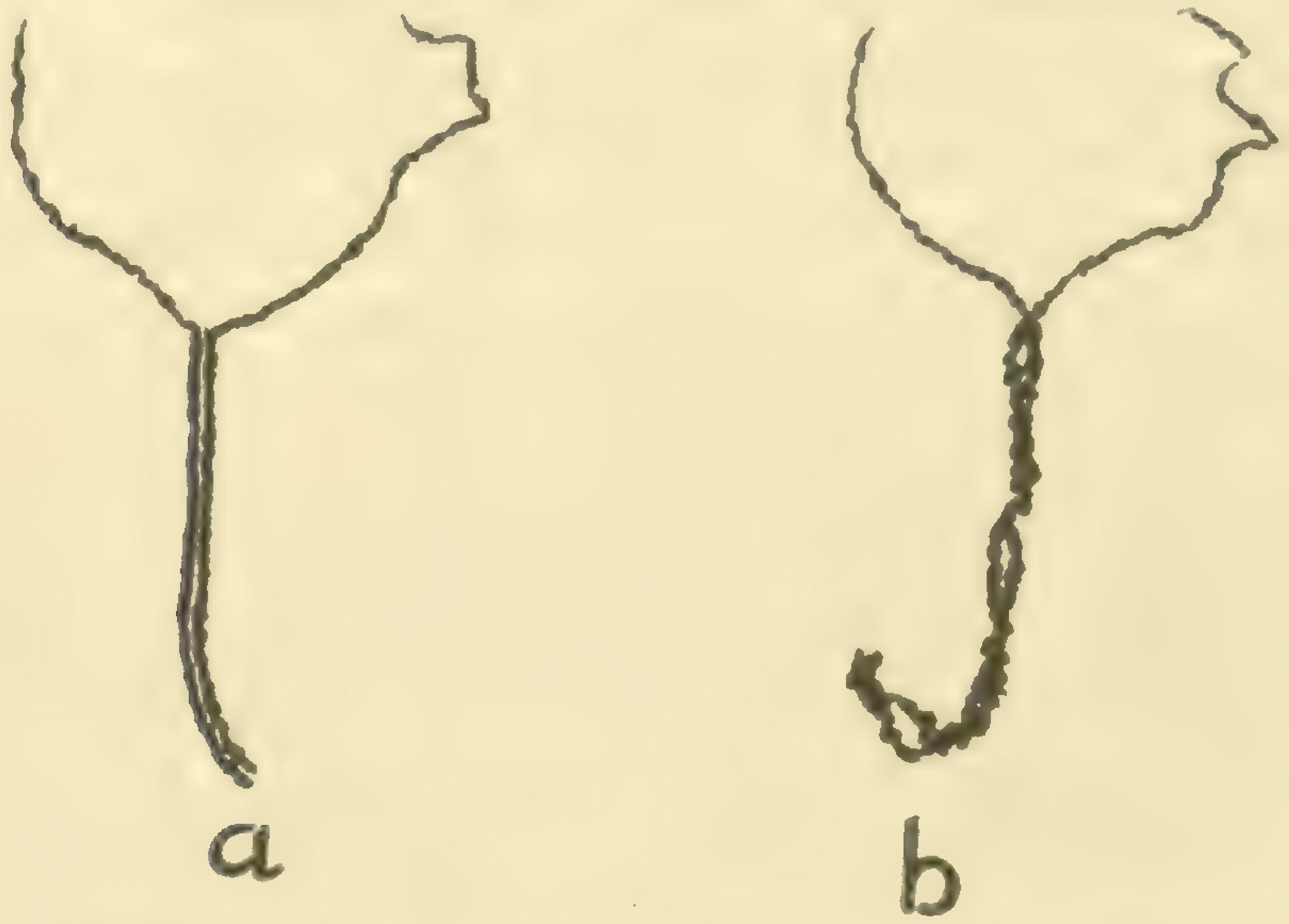

FIG. 6. Chromosomes during an early stage of synapsis (amphitene). In some preparations the apposed threads seem parallel, as in *a*; in others they seem twisted about each other, as in *b*.

long thread or "chromonema," which, during the metaphase and anaphase of mitosis, is coiled up very closely in more or less spiral fashion (probably within a viscous sheath of some sort), to form the thick dense chromosomes, but which, in the resting period and during the early stages of synapsis, becomes, to some extent at least, uncoiled and drawn out again. In this state, then, the chromosomes first pair, as shown in Fig. 6. Thus precisely homologous parts of the frail threads may become apposed to each other, so that this stage, which is called the "amphitene" stage, would seem to be the one best "adapted" for the occurrence of crossing-over. Later, when each chromosome becomes, presumably, a thick spiral, there would seem to be much greater mechanical difficulties in the way of exact apposition and breakage of parts.

On any possible theory of crossing-over, however, the known facts concerning interference should be capable of

interpretation. If crossing-over occurred during the "amphitene" stage, or not long after, would there be any possible explanation of the fact that one point of crossing-over is generally far removed from another? The explanation might be found simply in the fact that each of the "leptotene" chromosomes—*i. e.*, the finely drawn out chromosomes which are just about to undergo synapsis—pursued a general course that had few close turns in it. (For possibly it maintains the same general direction as it had when it was short and thick; the reader will recall that Boveri found that chromosomes preserve their approximate shape and position from one cell division to the next.) When, therefore, the leptotene chromosomes are being brought together by the synaptic attraction which homologous loci then bear for each other, the threads are usually crossed only at a few points, and these are generally far apart. If these initial points of crossing—which, it will be observed, have been determined by the original positions of the threads, and not by any twisting—are the points of crossing-over, interference would be accounted for, and would, in effect, be of the same general nature as on the mechanism of crossing-over postulated by Janssens.

It might at first seem hard to imagine why, on this second scheme of crossing-over, recombination—*i. e.*, "crossing-over"—should occur where the threads cross, but it should be remembered that the two threads, while coming together, often lie in about the same plane both above and below the point of crossing. If they keep to this original plane as they draw together, they will come to have the same plane of apposition just above and just below the crossing point,—although the sides of the filaments that face each other will be just the opposite in the two cases; consequently, the threads at the crossing point must undergo a very sharp twist, and if, as we must suppose, they are somewhat viscous, this may result in their breakage and recombination, or, perhaps, first in their fusion, and, later, when the pieces of the same chromosome above and below the point of crossing are wrenched

apart in opposite directions by mutual repulsion of the strands or by pulling of spindle fibres, in breakage of parts originally together. (So perhaps fusion might occur during the amphitene and breakage in the strepsinema stage; this would be a combination of schemes 1 and 2 which would account both for the exact apposition of parts and for the phenomena observed by Jannsens.) Be this as it may, at any rate, the negative argument may be given that it is just as hard to account for recombination at a later stage in synapsis as at this stage, even overlooking the objection of the thickness of the threads.

There is a serious objection to the scheme just given, however, in that, as the threads come together, they seem, in many preparations, not to keep their original plane of apposition, but to twist tightly about each other, like the strands of a rope, throughout their entire length (see Fig. 6). It is possible that the twisting of one thread about the other is merely apparent, however, and that the threads lie parallel but are simply coiling up in a spiral, in the process of forming the shorter, thicker prophase chromosomes; for, unless the spiral were very delicately preserved by the fixing agent, there would be apparent knots in it as though there were a twisting of two strands about each other. Moreover, there is evidence indicating that this tight twisting occurs only in certain species of animals. But let us assume for the moment that this very tight twisting really takes place during the amphitene stage in flies, and that crossing-over takes place at this period (this we may call scheme of crossing-over number three). Would there then be any way of explaining why one crossing-over should interfere with another near by, in view of the fact that the loops are of such small dimensions? In seeking an answer to this question, it will be helpful to bear in mind that crossing-over can be divided into just three essential processes—a bending of the chromosomes across each other, a breaking of the threads, and then a fusion of adjoining pieces (or, perhaps, the fusion of the homologous chromosomes comes first, and then the breaking of the original chromosomes at that

point). It follows from this that interference must in any case be due to one of the following three general causes: (1) Either the chromosomes are not likely to bend across each other twice at points near together (*i. e.,* the loop tends to be long), or (2) breakage at one point for some reason interferes with another breakage nearby (even though the threads are crossed at both of these points), or (3) fusion of chromosomes at one point in some way interferes with fusion of threads which are crossed in a neighboring region. That fusion at one point could interfere with fusion at another point can scarcely be imagined. And if crossing-over occurs according to scheme number three, the "loop explanation" must also be thrown out. Consequently, if crossing-over occurs at a stage of tight twisting the breakage of the threads at one point must somehow be considered to prevent another break near by. In explanation of this, breakage might be thought of as resulting from the tightness of the twisting, for then a breakage of the threads at one point would relieve the tension of the filaments for some distance along the line and so tend to prevent another breakage from occurring near by. (Later, when threads reunited at the point of breakage, pieces from homologous chromosomes would be as apt, or more apt, to lie end to end, and therefore to join, than pieces of the same chromosome. As a partial explanation of why the fragments should join again at all, it might be supposed that only the chromonemas break, the fused sheath which envelops the pair still holding the pieces together.)

It is fully realized that the above discussion is highly speculative. It is intended, however, not as a presentation of conclusions, but as a tentative suggestion of possibilities, in order to obtain some system of ideas that may furnish a temporary basis for a real attack—experimental and observational—upon the subject.

Tests for These Alternatives

Is there any way of obtaining evidence as to which of these three schemes of crossing-over is the more probable

one? Light might perhaps be thrown on the question by a closer study of interference, and it was largely for this reason that the experiment described in section V was undertaken. If, for example, interference was a result of length of loop (as would be true in schemes I and II), and the length of the loop tended to vary more or less in both directions, about a given mode, then coincidence would be relatively higher between crossings-over which were that distance apart, than between crossings-over nearer together or still further apart. In other words, as may be seen from Fig. 7, for small distances, the relative

FIG. 7. Diagram to illustrate the second scheme suggested for crossing-over. The amphitene threads become sharply crossed at a particular point.

coincidence would be very small (interference high), for longer distances much greater, and with still longer distances coincidence would fall again (interference would rise). For distances double or triple the length of the loop—if the chromosomes were as long as that—coincidence would rise once more. Secondly, on the "loop explanation" of interference just outlined, coincidence should, at the modal distance, rise above the 100 per cent. level, for crossing-over would occur at a given point (K) *more* often in those cases when there is crossing-over at another point (I) lying at the modal distance from K, than in the average case. Of course it might be, however, that there was no modal length of loop—that although short loops were infrequent, all loops above a certain size were equally frequent, or that the longer the loop, the more

frequent it tended to be. In the former case coincidence would rise to a certain level, as distance between the points of crossing-over considered increased, and would after that remain constant; in the latter case it would rise progressively, and might or might not reach or pass the 100 per cent. level.

On the other hand, if crossing-over is due to a breakage of tightly twisted threads, not so many different kinds of variation of coincidence, with increase in distance, would be theoretically possible, but a condition something like the one last mentioned must always obtain. For, on scheme 3, the interference of a breakage with the tightness of twisting and consequent chance for another breakage must decrease progressively at greater and greater distances from that breakage; coincidence would thus rise until finally it reached the 100 per cent. level expected on chance. It would never rise much[2] beyond this, as one break could never make another *more* likely to occur; neither could coincidence fall once more, with a still greater distance (as it could on the loop scheme, after a "modal distance" had been reached). If, therefore, it should be found that, for certain (modal) distances between two points of crossing-over, coincidence ran well above 100 per cent., or that, beyond certain distances, coincidence fell again, there would be good evidence that crossing-over did not occur at a stage of tight twisting. If, on the contrary, it were found that crossing-over coincidence rose progressively with distance, until it reached the 100 per cent. mark, but neither went much[2] beyond

[2] Even on scheme III, coincidence could finally rise slightly above 100 per cent., for although one break (I) could not help another (K) to occur, no matter how far away the latter (K) might be, still it might, by preventing the occurrence of other breaks (J), in between these two, give more chance for the occurrence of the break farther off (K), since in this way the interference of breaks J with K (which is stronger than the interference of the more distant I with K) is removed. Thus break K might occur more often when I also occurs than in the average case, and so coincidence would rise above 100 per cent. However, it would be easy to distinguish between the slight rise in coincidence above 100 per cent., due to this cause, and the rise which would exist on the loop explanation of interference if I and K were separated by a distance about equal to the modal length. For, in the first case, *considering only gametes in which no crossing-over at all took place in between*

this, nor fell again later, and if cytological measurements should then substantiate the judgment, based on inspection, that the loops did have a modal length during the strepsinema stage, there would be good evidence that crossing-over must occur at an early stage of synapsis.

Other peculiarities of coincidence also might be found which would permit of explanation on one scheme and not on another. In groups II and III, for example, there seem to be peculiarities in the coincidence relations in cases where the chromosomes differ in regard to the factor C, or a similar factor. And a comparison of coincidence in different regions of the chromosome in any given case or in the same region of the chromosome in cases of linkage variation, might very well reveal relations that lend evidence to one scheme of crossing-over or another. Even a determination, not of coincidence, but merely of linkage variation itself, in different parts of the chromosome, might in some way shed light on the subject. In the case of the third chromosome, experiments of this sort are now under way with multiple stocks which I have made up for this purpose, and Sturtevant is conducting similar experiments with group II. The first requirement, however, is obviously an accurate study of normal coincidence, and it therefore became necessary to determine the coincidence for points various distances apart, preferably in the same experiment. But to work with a great many factors in a group at once introduced new difficulties, which made special methods necessary, as will be explained later. Before considering this experiment, it will be desirable to consider other lines of evidence and modes of attacking the problem of crossing-over.

The cytological evidence which Janssens presents for crossing-over is entirely directed towards proving that crossing-over occurs during strepsinema or later. In strepsinema the chromosomes, as already mentioned,

(*at J*), it is easily seen that the proportion of breaks at K would be lower when breakage occured at I than when there was no breakage at I, whereas in the second case, the proportion of breaks at K would in such gametes be higher when there was breakage at I than when there was no breakage at I.

become much shorter and thicker than in the amphitene stage, and each chromosome in the pair can in many preparations be seen to have split lengthwise, *i. e.*, the "tetrads" have formed preparatory to the two maturation divisions. Janssens often finds the four threads placed somewhat as shown in Fig. 8*a*, two of the threads crossing at one or two points, but otherwise being rather widely separated, and the other two threads rarely crossing but lying close to whichever one of the two threads first mentioned happens to be on the same side, and merely bending inwards and then back again where the first two threads cross. The peculiar crossing of two of the threads and the bend in the other two, as shown at point L, he interprets, in the way shown in Fig. 8*b*, as meaning

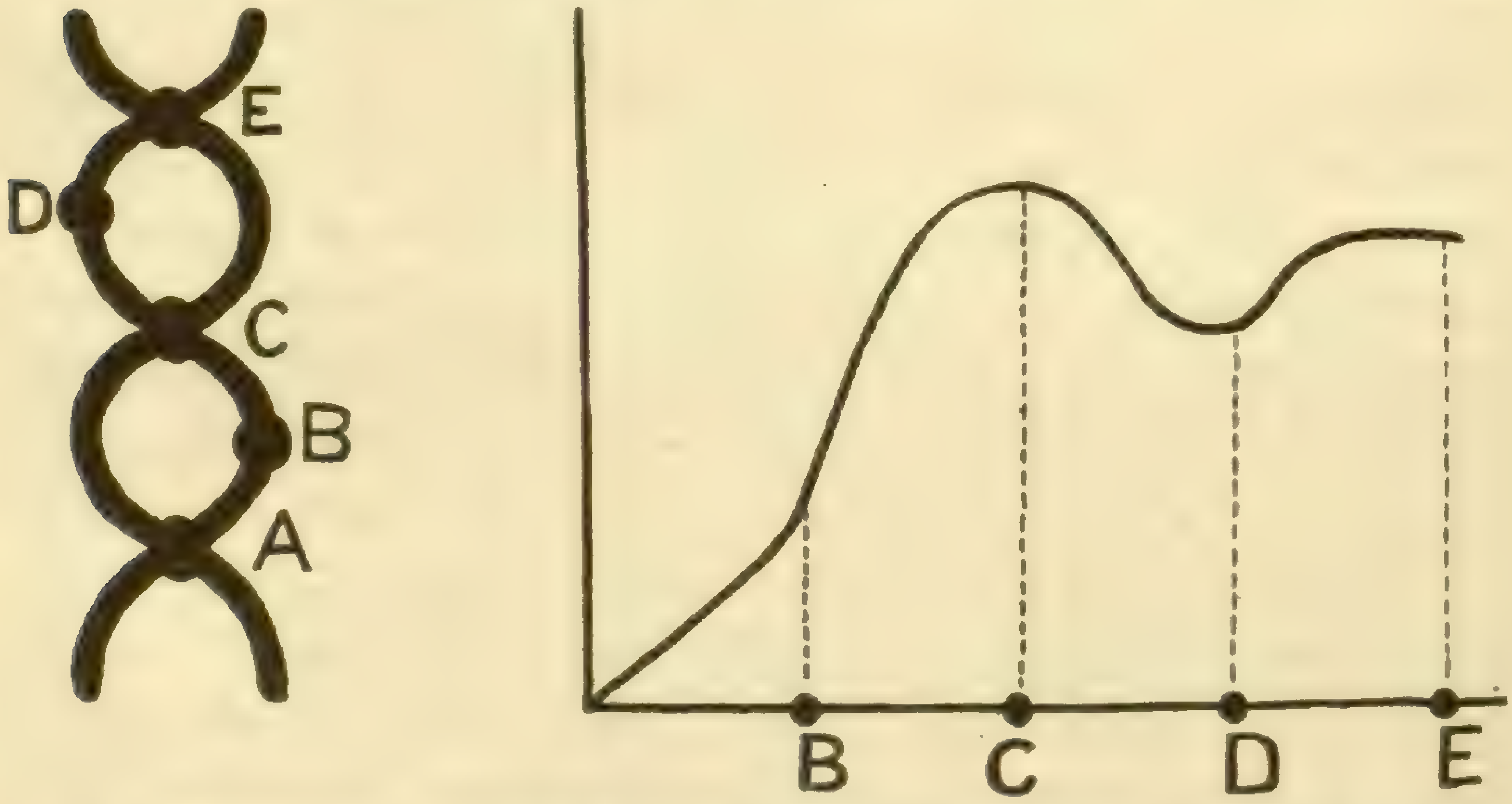

FIG. 8. Diagram to show possible coincidence relations on schemes I and II. The chromosomes are represented as crossing-over at A, and twisting in loops of the most usual (modal) length. It will be seen that a crossing-over at A will rarely coincide with one nearby—at B—since then the chromosomes would have to twist in loops much smaller than the modal length. But it will often coincide with one at C, seldom with one at D, and often again with one at E. The *relative coincidence* of crossings-over at various points on this chromosome with crossing-over at A is shown in the curve on the right.

that both pairs of threads originally were twisted across each other, but that the two homologous threads which were originally on the inner side, and so touched each other, underwent recombination, *i. e.*, "crossed over," at the point of contact; each of the new chromosomes thus formed, therefore, would lie entirely on one side or the

other; the other two threads, on the contrary, are supposed not to have undergone recombination ("crossing-over") and therefore would still lie across each other.

It would seem equally possible, however, to interpret these figures as meaning that (as shown in Fig. 9*c* and 9*d*) when the four threads began to separate into two pairs, separation happened to start at some points (A and C) between the identical halves and at other points (B) between the homologous chromosomes, it being merely a matter of chance in which way the separation started to take place. It will be seen that this would result in the formation of just such cross-figures, between two regions where separation took place in opposite ways, as Janssens finds.

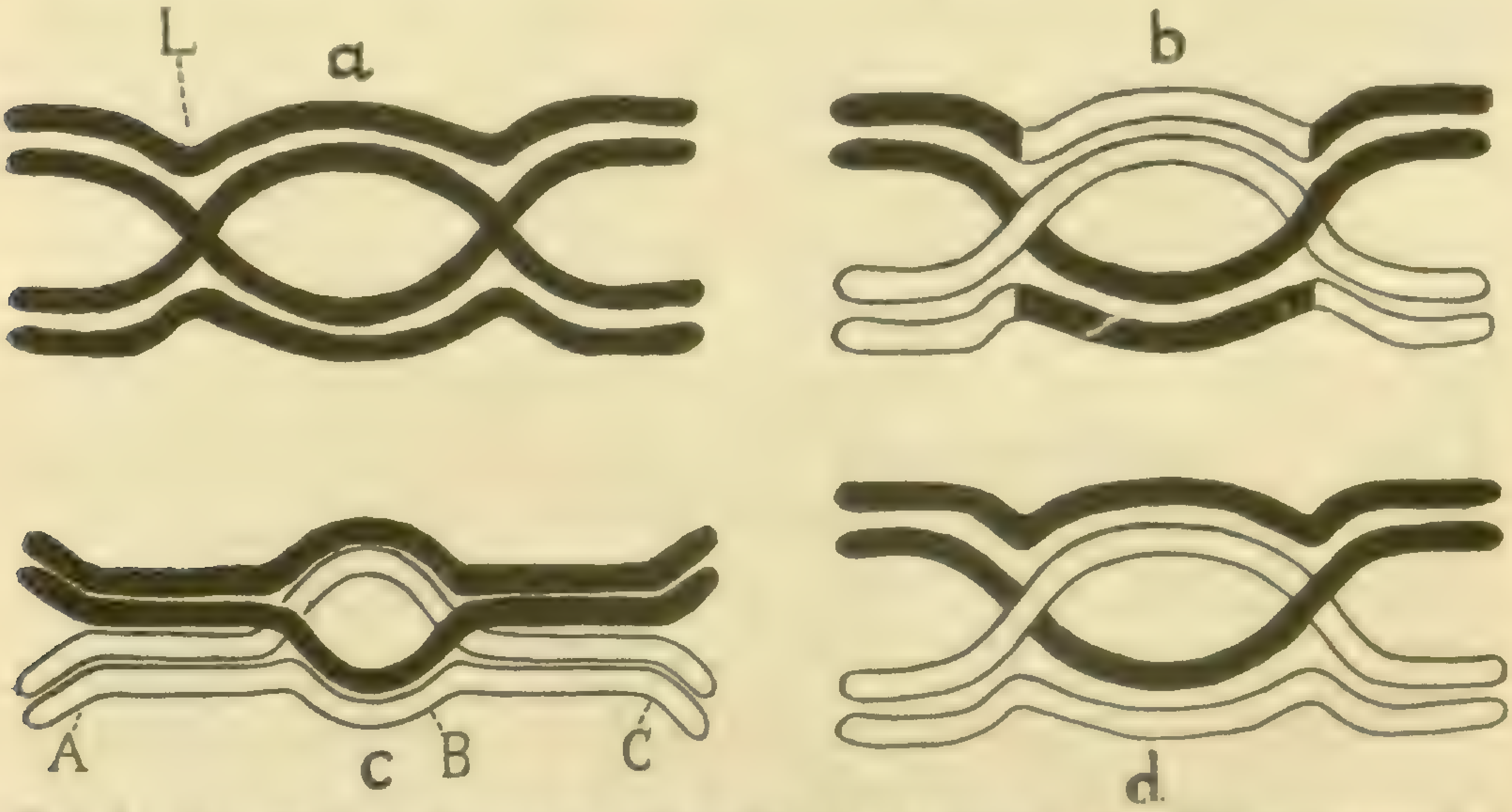

FIG. 9. (*a*) The chiasmatype described by Janssens. (*b*) His interpretation of it. (*c*) and (*d*) A suggested alternative interpretation.

Another point in Janssens's evidence is that the prophase chromosomes of maturation divisions not only show the strands crossing, at points, but often bending in towards each other near the middle, as though they had formerly crossed there, and later undergone crossing-over. It would seem possible, however, that this figure is merely due to the chromosomes remaining in contact more closely at the point where the spindle fiber is attached, and spreading apart elsewhere,—a relation which figures of Bridges and others show to exist between the two identical halves of chromosomes in the prophases of oogonial mitoses.

Finally, Janssens says that crossing-over is indicated by the fact that the chromosomes often seem to have sunken into one another at the crossing point. This detail, which would be very difficult to establish, might, of course (if it had any significance at all), merely mean that the chromosomes were still closely attached at the point where they had *previously* crossed-over. It seems incautious, therefore, to regard the cytological evidence as showing more than the possible means of the crossing-over which the evidence from factor and chromosome distribution demonstrates to occur.

Some of the Orthopteran material which gives such clear-cut chromosome figures might perhaps settle the point whether crossing-over occurs at the stage of four threads, as Janssens believed. For it is reported by Wenrich (19) that homologous chromosomes can sometimes be distinguished from one another in prophases by differences in the size or shape of contained granules, that are constant for the particular individual. Moreover, the four threads are clearly distinguishable in the prophase of the first maturation division. If a female could be found (there is some reason to believe that crossing-over does not occur in the male) which showed a difference in respect to two granules at different points in the same pair of chromosomes, then, if Janssens's theory is right, it would happen that, in some of the oocytes, of the four post-synaptic threads two would have a new combination of granules and the other two would not show any interchange. But on the view that crossing-over occurs earlier —the identical halves being formed after interchange has taken place—all four threads would be of a new combination in those cases where crossing-over of chromosomes in the region between the two pairs of granules had occurred at all.

There is an essentially similar possibility of finding out the same thing genetically. For if two threads may cross over and not the other two, then, if non-disjunction of X-chromosomes should occur in that maturation division

when the threads that crossed would normally have separated from those that did not, the egg would come to contain two X-chromosomes, one of which was a cross-over but not the other. In the usual type of non-disjunction, the X's never cross over—presumably because they paired with the Y (which was present in these cases as an extra chromosome), so this type of non-disjunction could not afford a test of the theory. But it is to be expected that non-disjunction should sometimes occur by mere accident without the interference of a Y, and since in these cases the X's could have crossed over, such cases of non-disjunction might furnish a test of Janssens's theory. In 1913, in an experiment designed for this purpose, I obtained a fly which had received two maternal X-chromosomes by reason of non-disjunction in its mother, and in which one of these X-chromosomes proved to be a cross-over but not the other! The fly resulted from a cross of a female which contained in one X-chromosome bifid and vermilion, and in the other chromosome eosin and bar, by a normal male. It itself contained in one of its chromosomes bifid and vermilion, and in the bifid, vermilion and bar. Since then Bridges has obtained other exceptions of the same general sort. But on further consideration it appears that this result really proves nothing, for the non-disjunction may just as well have taken place in an oogonial division. In this way an oocyte would result that contained three X-chromosomes. At synapsis two of these could cross over with one another, and the egg could then receive a cross-over chromosome and also an X that had not crossed over. To prove that the non-disjunction was not of this type, but really occurred in a maturation division, *i. e.*, that the two threads originated from one tetrad, it would be necessary to obtain individuals in which both of the X-chromosomes received by non-disjunction had crossed over, but each at a different point (or one of them at two points).

In a case of the latter sort the fact that both chromosomes had crossed over at some point would prove either that both of them had been in the synaptic tetrad, and so

that the non-disjunction had occurred in the maturation division in the mother fly, or else that both were derived from the two halves of a single (cross-over) X-chromosome which underwent non-disjunction in an embryonic cell division of the individual itself. But the fact that the two chromosomes are not identical would rule out the second possibility. The result, therefore, would mean that in the same tetrad one strand may have crossed over at a certain point and not another strand, *i. e.,* that Janssens's theory is correct and crossing-over takes place at a stage when there are four threads, two of which may cross over at a certain point while the others retain their original composition.

Up to the present, however, no exceptions of this type have been found, although Bridges has obtained not a few exceptions of the type that may as well be explained by non-disjunction in an oogonial division (*i. e.,* in which one X had crossed over—but not the other), and also one other exception, which had received two similar double cross-over chromosomes. The latter peculiar circumstance must have resulted either from a non-disjunction, at the maturation division in the mother, of two strands of a tetrad, both of which had crossed over in the *same two* places, or from a non-disjunction, in an embryonic cell division of the individual itself, of the two halves of the single (double cross-over) X-chromosome, which, on this view, was originally present. But the latter explanation is very improbable, for, unless the non-disjunction occurred in the first cleavage, only a small part of the fly would be composed of cells descended from the one into which the 2 X's entered; most of the cells, therefore, would contain only one X and these would necessarily be male; thus the fly would be a gynandromorph. Moreover, all the cells derived from the one which, in the non-disjunctional division, failed to receive either half of the X-chromosome, would probably die. Hence the evidence is fairly good that in this case the two double cross-over X-chromosomes represent two strands of a tetrad. Since these two strands, although both double cross-overs, were

both just alike, we must conclude either that they were both derived from the same strand, after it had already crossed over—in which case crossing-over must occur at a stage in synapsis before the homologous chromosomes split to form tetrads—or else that the tetrads were formed first, and that then crossing-over occurred at two points coincidently in the case of both pairs of threads, and at identical points in both. It is not probable, however, that, if crossing-over occurs at the stage of four threads, these two pairs of threads would both cross over at the same points, for according to the observations on which Jannsens bases the idea that crossing-over occurs at this stage, a crossing-over of both pairs of threads at the same place rarely happens. The evidence thus far gained from non-disjunction is, therefore, rather in support of the theory that crossing-over occurs at an early stage in synapsis.

D. A Case of Crossing-Over in an Embryonic Cell

It may not be out of place here to record an exceptional case of crossing-over in the male, which has not been explained. No other case of crossing-over has hitherto been found in the male Drosophila. It had been established by Altenburg and the author that the factor causing truncate wings is in the second chromosome, and further that the truncate factor is dominant under certain conditions, but it does not usually express itself unless certain intensifying factors—one in the first chromosome and one in the third—are present; even then, the character sometimes fails to develop. Thus, if a hybrid truncate male is produced by a cross of a truncate female to a black pink male (black is in chromosome II and pink in III), when this hybrid is back-crossed again to black pink females, only the gray flies will carry the factor for truncate, since in the male truncate can not cross over with the black in the homologous second chromosome. But few of the gray flies from such a cross except the gray, red-eyed females will show the truncate character, for the others will not contain both of the intensifying factors; and even in the

gray-red females the character will not always develop.

A typical count for such a cross was as follows:

Gray Red			Gray Pink			Black Red			Black Pink		
Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.
57	23	7	2	14	64	0	0	74	0	0	82
2	29	47	1	8	62	0	0	67	0	0	73

(The count of females is shown on the upper line, the count of males on the lower.) A brother of the above male, however, when similarly back-crossed, gave the following count:

Gray Red			Gray Pink			Black Red			Black Pink		
Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.	Trunc.	Inter.	Norm.
0	0	32	0	0	19	16	20	1	0	18	7
0	0	15	0	0	23	0	6	11	0	3	22

The sex-linked intensifier and the third chromosome intensifier are inherited normally as before, for the females have wings much more truncated than the males and the reds are more truncated than the pinks. But, although the truncate parent of this male contained gray in the same chromosome as truncate, and the long-winged parent contained black with long, all the truncate has crossed over, away from the gray factor and into the chromosome with black! Not a single fly has the old combination, gray truncate. It is next to impossible to imagine that the chromosomes of the second pair crossed over in the synapsis period of all the spermatocytes, and in all of them, between just these particular loci, when normally there is no crossing-over at all in the male and only 30 per cent. of crossing-over between these loci even in the female. It is, therefore, necessary to conclude that crossing-over took place once for all in a cell of the embryo, and that, as usual, it did not occur at all during spermatogenesis, although all the spermatocytes, of course, inherited the cross-over combination. It is impossible to tell whether or not the chromosomes underwent the regular process of synapsis at this early stage,

and whether they crossed over when long drawn out or when short and thick, but at least the fact remains that crossing-over may, in abnormal cases, take place in a cell before the definitive growth period is reached, and even in an individual (*Drosophila* male) in which no crossing-over is the established rule. This fact is not utterly surprising, inasmuch as even in somatic and gonial cells of Diptera homologous chromosomes show a marked tendency to lie near together (*i. e.*, to attract each other), and in Metz's preparations they may not infrequently be found even twisted about each other somewhat.

The fact that crossing-over occurs only in the female *Drosophila* is naturally of great interest, although it is of unknown significance. In the silkworms, on the other hand, Tanaka has discovered that crossing-over takes place in the male, but not in the female. Curiously enough, although these seem at first sight to be opposite cases, in both it is true that crossing-over takes place in the homozygous sex, but not in the heterozygous, for in *Drosophila* the female is homozygous for sex, the male heterozygous, and in the moth these relations are reversed. Recently, however, Castle and Wright have published data for the rat which, if sufficiently extensive, show that crossing-over happens in both sexes. The plants in which crossing-over has so far been studied have all been hermaphrodites, and crossing-over takes place in both their spermato- and oo-genesis. There is, therefore, at present no general rule which can be stated, in regard to which sex crossing-over occurs in. This fact should be taken into account in weighing the cytological evidence in regard to crossing-over, obtained in forms in which the occurrence of crossing-over has not been studied genetically. For in such cases there is always the possibility that the cytological studies are being conducted on individuals in which crossing-over does not occur and which would consequently give results quite irrelevant to the subject.

(*To be continued*)

FASCIATION IN MAIZE KERNELS[1]

T. K. WOLFE

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In the summer of 1914 a number of different varieties of corn were crossed for the purpose of studying the effect of hybridization on the weight of hybrid and pure seed produced. One of the crosses made was between Improved Leaming as the seed parent and Boone County Special as the pollen parent, the pollen of the two varieties being mixed and applied to the same ear. The former variety is a yellow dent and the latter a white dent. On this ear was found two kernels, each of which had two embryos. The description of the kernels and their progeny will be given in this paper.

Description of Kernels

In corn, the embryo is normally on the side of the kernel toward the tip of the ear. These kernels had an embryo on both sides. The kernels seemed to be normal with the exception of the extra embryo and a slight prominence or line of demarkation which extended around each kernel parallel to the embryos.

Kernel No. 1 was yellow in one half, while the other half was a paler yellow (diluted with white). Kernel No. 2 was yellow in both halves. Although there was a variation in the degree of color, the results of the F_1 generation proved that both halves of each kernel were hybrid.

Progeny from Kernels

The kernels were planted in pots in the greenhouse in April in greenhouse soil and in due time each kernel pro-

[1] Paper 2 from department of agronomy, Virginia Agricultural Experiment Station, Blacksburg, Virginia.

FIG. 1. F_1 generation progeny of fasciated kernels. Stalks on the left, progeny of kernel No. 2, those on the right, progeny of kernel No. 1. (About one fifteenth natural size.)

duced two stalks. In May, after danger of frost was over, the contents of each pot were removed from the greenhouse and placed in the field. The time of tasseling and silking and other data were recorded during the season as shown in Table I. Each stalk produced two ear shoots; however, only one ear shoot on each stalk produced an ear.

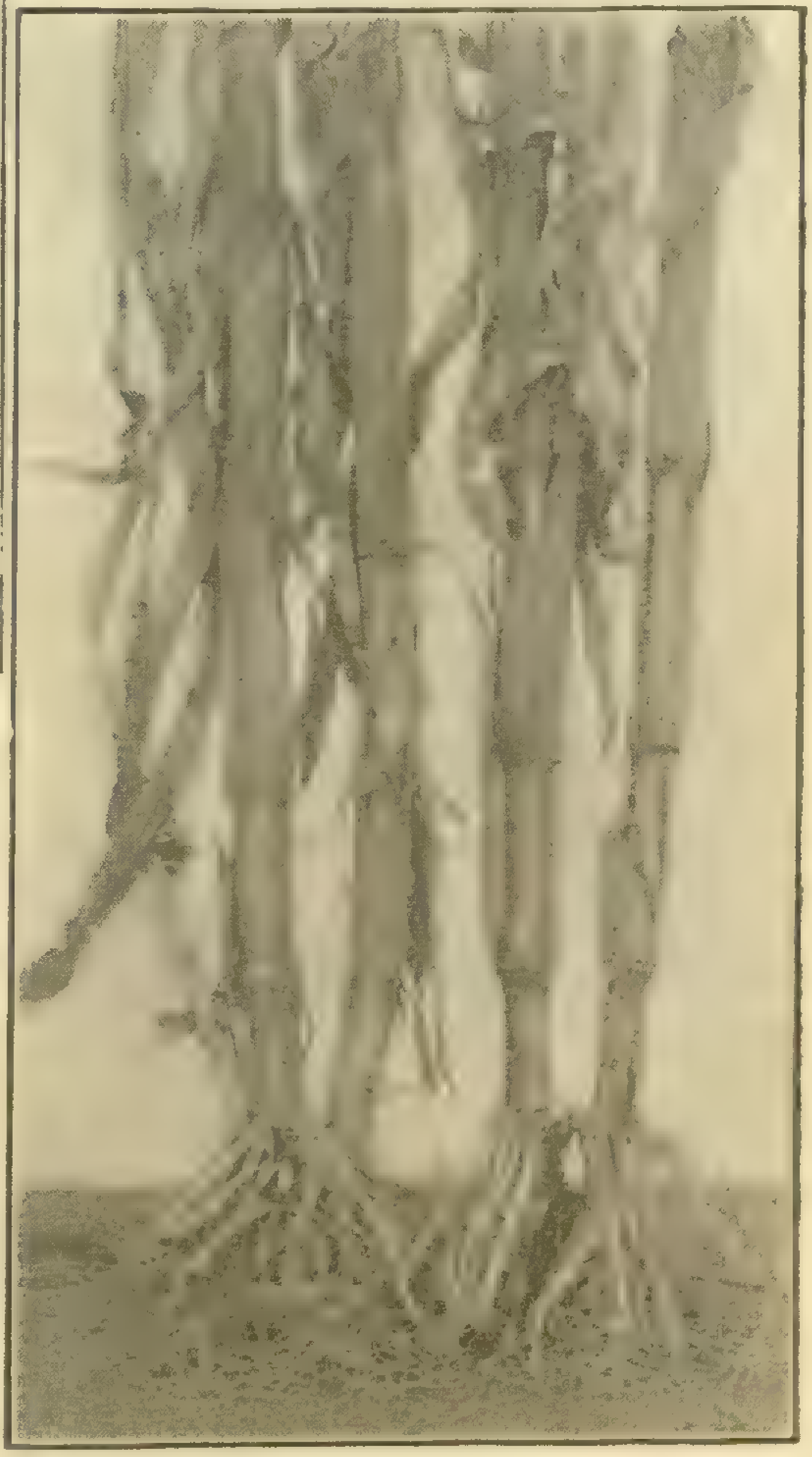


FIG. 2. F_1 generation progeny from fasciated kernel No. 2 on the left, and fasciated kernel No. 1 on the right, showing root systems. (About one fifth natural size.)

At first the tassels and silks were bagged to prevent foreign pollination. All the pistillate flowers were self-pollinated, the pollen being applied by hand at this time. Later, paper tubes were fastened to the tassel and carried to the uppermost ear shoot, the lower ear

shoots being covered with bags and hand pollinated as was done at first.

DESCRIPTION OF F_1 GENERATION STALKS

After the total growth had been made, data were recorded as to the height and diameter of stalks, length, width, and number of leaves, while the dates of tasseling and silking had been obtained previously.

Fig. 1 shows picture of entire stalks after harvesting.

TABLE I

HEIGHT AND DIAMETER OF STALKS, LENGTH AND WIDTH OF LEAVES, IN INCHES. NUMBER OF LEAVES AND DATES OF TASSELING AND SILKING OF F_1 GENERATION STALKS FROM FASCIATED KERNELS OF MAIZE

Kernel No.	Stalk No.	Number of Leaves	Height of Stalks	Length of Leaves		Width of Leaves		Diameter of Stalk	Date of Tasseling	Date of Silking
				8th	9th	8th	9th		July	
1	1	11	99	$33\frac{1}{4}$	$31\frac{7}{8}$	$3\frac{15}{16}$	$3\frac{13}{16}$	$\frac{11}{16}$	12	26
	2	13	102	$36\frac{1}{8}$	$33\frac{1}{2}$	$4\frac{1}{16}$	$3\frac{3}{4}$	$\frac{3}{4}$	16	24
2	3	14	$92\frac{1}{4}$	$36\frac{3}{4}$	$34\frac{5}{8}$	$4\frac{11}{16}$	$4\frac{5}{8}$	$1\frac{1}{8}$	12	19
	4	14	$108\frac{3}{4}$	$40\frac{1}{8}$	$37\frac{5}{8}$	$4\frac{1}{16}$	$4\frac{3}{16}$	$\frac{7}{8}$	14	21

At maturity, the entire plants were removed from the ground in such a way as to retain as many of the roots as possible. The soil was removed and a photograph was taken of the roots (Fig. 2) to especially emphasize the fact that each stalk was separate and distinct from the other and could not be classed as a tiller from the other stalk, although both were united at the radicle.

DESCRIPTION OF F_1 GENERATION KERNELS

Fig. 3 is a photograph of the four ears produced. All of them show Mendelian splitting. The number and ratio of yellow and white kernels will be given in Table II. None of the kernels possessed two embryos like their parents.

FIG. 3. F_1 generation ears produced by the fasciated kernels. Beginning at the left, the first and second ears were produced by kernel No. 2; stalks numbers 1 and 2, respectively. The third and fourth ears were produced by kernel No. 1; stalks 3 and 4, respectively. (About one half natural size.)

TABLE II

NUMBER AND RATIO OF WHITE AND YELLOW KERNELS IN THE F_1 GENERATION

Kernel No.	Stalk No.	Ear No.	Number Yellow Kernels	Number White Kernels	Ratio of Yellow to White Kernels
1	1	1	218	76	2.86:1
	2	2	377	60	6.28:1
2	3	3	393	182	2.15:1
	4	4	408	130	3.14:1
					Average ratio, 3.61:1

This generation seed will be grown next season in order to discover whether any fasciated kernels appear. After these results are obtained, a discussion of the kernels reported in this paper will be presented.

SHORTER ARTICLES AND DISCUSSION

THE INHERITANCE OF SEASONAL POLYMORPHISM IN BUTTERFLIES

ARE seasonal variations inherited, and may they play a part in evolutionary change? These are questions which Punnett in his recent book on "Mimicry in Butterflies" answers in the negative.

> In no case are they known to be inherited, and in no case consequently could variation of this nature play any part in evolutionary change.

Variations to be of significance in evolution, he tells us, must be "transmissible and independent of climatic and other conditions."[1]

It would seem to require no demonstration that well-established seasonal variations like those of *Araschnia levana-prorsa* of Europe in which, it will be remembered, the ground color of the spring brood (*levana*) is red-brown, that of the summer brood (*prorsa*) black, are transmissible. Under summer conditions in Europe *prorsa* appears with the regularity of a monotypic species, true to type. Monotypic species likewise require a certain degree of temperature and amount of moisture to produce their characteristic adult coloration. *A. prorsa* is by no means peculiar in this respect. It has the definitive adult coloration of the species. That which is peculiar is the hereditary rhythmic tendency to swing from *prorsa* back to *levana*, which is so strong that experimental control can not wholly cope with it. Summer conditions artificially prolonged result in the appearance of some *prorsa* in *prorsa's* immediate offspring, but sometimes the intermediate, *porima*, is the outcome. A far larger number of individuals of the lot under experimentation, however, refuse to be forced out of the chrysalis by artificial heat, hibernate, and become *levana*. These color variations, therefore, are not subject wholly to the environment, nor wholly to heredity.

A common hereditary basis evidently underlies both of the

[1] Pp. 131, 132.

color patterns. Like produces like, but under natural conditions only by skipping a generation. Except for the innate tendency for the types to alternate, the case is similar to that of the red primrose described by Baur[2] which, growing at 15°–20° C. produces red flowers, at 30°–35° C., white. Or it is like the mutant stock of *Drosophila* described by Miss Hoge,[3] which, bred in winter or in an ice chest, gives a large proportion of flies with supernumerary legs, though in summer or in moderate temperature the stock appears to be normal. The same set of factors under varying conditions produces different results. An analysis of the factors underlying another similar case in *Drosophila*, "abnormal abdomen," has been worked out by Morgan.[4] This remarkable mutant was shown to behave as a dominant sex-linked character. It manifests itself, however, only when the food in which the flies are bred is kept moist.

The rhythmic tendency of *prorsa* to produce *levana*, notwithstanding artificial raising of the temperature, shows that this is a sort of alternation of generations in which the definitive sexual generation, *prorsa*, alternates with another apparently more primitive, *levana*, which is also sexual. This seasonal alternation of sexual forms in its hereditary basis is comparable to typical alternation of asexual and sexual types.

Weismann,[5] in discussing the case cited, assumed the presence simultaneously in the germ plasm of *prorsa*-determinants and *levana*-determinants.

> But these *prorsa*-ids were at the same time so arranged that they became active under the action of a higher temperature, if this is acting at the beginning of the pupal period, while the *levana*-ids become active at a lower temperature. Heat, therefore, is only the excitant which sets free the *prorsa*-determinants, while cold sets free the *levana*-determinants.

Modernizing Weismann's hypothesis, may we suppose that distinct Mendelian factors underlie each of these two discontinuous types of coloration? The idea is attractive, but all the evidence at hand indicates that the determinants or factors of both types are borne by all the gametes. Intermediates, showing a

[2] "Einführung in die Vererbungslehre," pp. 4–6.

[3] *Jour. Exper. Zool.*, 18, 1915.

[4] "Mechanism of Mendelian Heredity," pp. 39–41.

[5] "New Experiments on the Seasonal Dimorphism of Lepidoptera." Translation by Nicholson in the *Entomologist*, 1896.

combination of the two patterns, called *porima*, occur under certain temperature conditions. The two patterns can not be Mendelian allelomorphs of each other, though the possibility remains they may have undiscovered allelomorphs. Too little is now known of inheritance in this species for us to judge whether Weismann's hypothesis in modern form is tenable, or whether a single set of factors, or single factor, reacting differently to different environments, is sufficient to account for the two types.

In a preliminary analysis of the problem the two color phases seem like distinct ontogenetic stages. *Levana* possibly is *prorsa* with immature colors, arrested in their development through the action of cold. *Prorsa* in the chrysalis may pass rapidly through the *levana* stage into its final, complete condition. Its offspring, however, independently of the environment, hereditarily tend to hibernate in the chrysalis and become *levana*. This interpretation of the two color phases is in line with the facts of dichromatism in beetles, as described by McCracken[6] and others. *Gastroidea dissimilis*, when it emerges from the pupal case, is black, and certain individuals permanently retain this color, others, however, pass on to a permanent bright green phase. *Lina lapponica* (*Melosoma scripta*) has a spotted-brown phase which is either permanent or is replaced by black. The two color phases in each of these forms, however, are Mendelian allelomorphs of each other, the dominant color being that appearing first in ontogeny, the recessive last.

The cold weather varieties of *Colias eurytheme*, about to be discussed, certainly may be regarded as being produced in large part by the arrested development of pigmentation. In this most remarkable seasonally polymorphic butterfly of western and central North America, *Colias eurytheme*, the writer has found that the flaming orange coloration of the summer form (usually called the typical *eurytheme*) and the paler orange-yellow of the spring and autumn broods (*ariadne* and *keewaydin*) are variations also due to differences in the reaction to the environment of definite Mendelian factors. This has been shown by crossing the orange *eurytheme* of the central and western states, with the clear yellow species of the eastern and central states, *Colias philodice*, the yellow of which segregates cleanly from the orange in F_2, as a recessive. The hybrids, as well as the *eurytheme* stock, show seasonal polymorphism. The F_1 hybrids, for example, are of a

[6] *Jour. Exper. Zool.*, 3, 1906.

dilute orange. Orange is therefore incompletely dominant. The heterozygote is an intermediate. The amount of orange pigmentation, or the degree of its dilution, in the F_1 hybrids, however, varies prodigiously with the season. The summer-bred hybrid is of dilute orange ("apricot yellow") somewhat evenly distributed over the wings, but in the small winter-bred individuals, such as emerge in the greenhouse in December, the orange is restricted to a faint flush near the posterior (inner) margin of the fore wings.

But even though the underlying hereditary basis supports a superstructure that varies widely, are these variations *as such* inherited? The variety of *eurytheme* called *ariadne* is small and of a pale orange hue. This form appears under cold weather conditions only. Its dwarfness is due to the failure of the caterpillar to feed during the late fall to full size, though food is abundantly supplied. The shortness of the day evidently is a factor in checking the feeding. The caterpillar forages actively at mid-day, but becomes sluggish before nightfall, yet it matures even while it is not feeding, and hence produces a dwarfed pupa. The pale color may be readily explained by the supposition that the elaboration of chromogenic substances in the blood of the pupa is checked by the cold so that these materials ripen in the scales of the wings merely into faint orange and yellow.

Ariadne's progeny in June are not *ariadne*, but a large and brilliantly orange insect. Are *ariadne's* size and hue, therefore, not inheritable, but dependent wholly upon the environment? At first thought this would seem to be the fact, and this was evidently the view of the matter presenting itself to Punnett when he stated that such variations are not inherited. We have seen, however, that they are hereditary in the sense that this organism must react in this particular way under these particular conditions. Its inherited organization compels, determines, this reaction.

These seasonal variations are therefore transmissible, though they are by no means "independent of climatic and other conditions." May they, therefore, play no part in evolutionary change? We should not yet be dogmatic as to this. The underlying hereditary basis in all probability is as susceptible to mutation, to disturbances in the arrangement and nature of the chromosomal elements, as any other germ-plasm. It would seem by no means impossible that the alternating phenotypes of *A.*

levana-prorsa, for example, might in suitable diverse climates, subarctic and tropical, respectively, be fixed as separate species. *A. levana* bred in Labrador, for example, where it could produce only one brood, probably would not show its *prorsa*-producing tendency at all. This supposition is confirmed by Trybom's observation (quoted by Weismann) that in Siberia, where a single brood occurs yearly, it is *levana only.* Conversely, *prorsa* in the tropics would perhaps eliminate all traces of *levana,* though of this we can not be so confident.

How much practically identical germ-plasm in different parts of the world is masquerading as different species, because of the diverse ways in which it reacts to different environments in which it happens to be placed, has not been adequately investigated. An interesting example of the sort among tropical reef fishes was recently cited by Longley.[7]

Bodianus fulvus and *B. punctatus* are two color phases of one species of which one may almost instantaneously replace the other.

The rapid interchange of reproductive habits between *Salamandra maculosa* and the alpine *atra* when transferred respectively to lowland or highland conditions, as described by Kammerer,[8] is probably also a case in point, due to fundamentally similar germ-plasm in both forms. Such an assumption would account for the inheritance of these readily acquired characters. If the facts are correct, *S. maculosa,* by cold and drought, was forced to assume the reproductive habits of the salamander of the neighboring alpine regions named *atra,* producing two adult larvæ viviparously, rather than many (14–72) immature embryos laid in water as is its habit in the warm, moist lowlands. Conversely, the alpine form was forced by heat and an ample water supply to increase its fecundity from two to nine larvæ at a birth. In both cases the "acquired characters" were inherited, as we would expect them to be if the two kinds of salamanders, as regards reproductive mechanism at least, have an identical or similar genotype. The fact that the lowland form living at higher altitudes has fewer young, and that the alpine form in the lower regions of its range produces an abnormally large number (viz., four) also points to the same conclusion.

It is a possibility worth considering that somatic modification accompanied by little germinal change may partially explain

[7] Carnegie Institution of Washington, Year Book, No. 14, 1915, p. 209.

[8] *Archiv f. Entwicklungsmechanik,* 25, 1907.

the remarkable "mimicry-rings" of South America that have been so interestingly discussed by Punnett. In each of several great regions of that continent a characteristic color pattern is exhibited by unrelated species belonging to different genera and even to different families. The color pattern followed in Central America differs slightly from that adopted by members of the same genera in eastern Brazil, a single genus of Pierids only dropping out of the ring in the latter region. In western Brazil and the upper Amazons the pattern is somewhat more mottled and the ground color darker, but the same genera are represented almost without exception. Finally, in Ecuador, Peru and Bolivia the pattern common to the different genera is still darker and greatly simplified. The Pierids here have left the ring, and a *Papilio,* an *Acræa* and two species of the Satyrid genus *Pedaliodes* have entered it. The point to be emphasized, however, is that the same genera, *e. g., Heliconius, Mechanitis,* have representatives in each local color group.

Let us now assume, with Punnett, that a set of similar or identical color factors is common to all the structurally diverse members of each ring, and add the further hypothesis that the color pattern resulting from these particular factors is to a large extent influenced by climatic conditions, as in seasonally polymorphic insects. It then follows that certain members of a "ring" migrating from the tropical climate of Brazil to the temperate zone farther south, even before they should become changed genotypically, would react to the new environment by assuming a new color pattern such as that now characteristic of the south temperate zone. If the genotype were identical throughout the migrating group and a single member of the group should so react, all naturally would respond in the same manner. Seasonal polymorphism thus may furnish an additional clue to the explanation of this most interesting case of convergence and parallelism in evolution.

This discussion leads to the conclusion that seasonal variations have a hereditary basis more sensitive than that of other color characters to temperature and other climatic conditions. A seasonal variation that is constant in its recurrence is transmissible. Its hereditary basis invariably reacts in a certain definite way to a certain narrow range of external conditions, whereas the hereditary basis of other characters, *e. g.,* eye color in vertebrates,

reacts in a precise way to a far wider range of external conditions.

Seasonal variations, as was pointed out by Weismann, show a hereditary tendency to alternate which, in some cases, is independent of external conditions.

Seasonal varieties are in some cases (*e. g.*, *Colias*, and possibly *Araschnia*) to be regarded as distinct ontogenetic stages. Cold arrests development at an early phase in color metabolism, and the mature insect emerges with pale colors (*Colias eurytheme* var. *ariadne*), or with a color pattern different from the definitive coloration of the species (*Araschnia levana*).

The suggestion is made that local color varieties, passing it may be for distinct species, are probably in some cases the equivalents of seasonal variations. That is, they are the product of a genotype sensitive to environmental changes expressing itself under a particular set of local climatic conditions; elsewhere the same genotype may respond quite differently. Such phenomena, though not of profound evolutionary significance, may play a rather conspicuous rôle in the evolution and diversification of the colors of animals and plants.

JOHN H. GEROULD

VARIATIONS IN THE VERMILION-SPOTTED NEWT, D. VIRIDESCENS

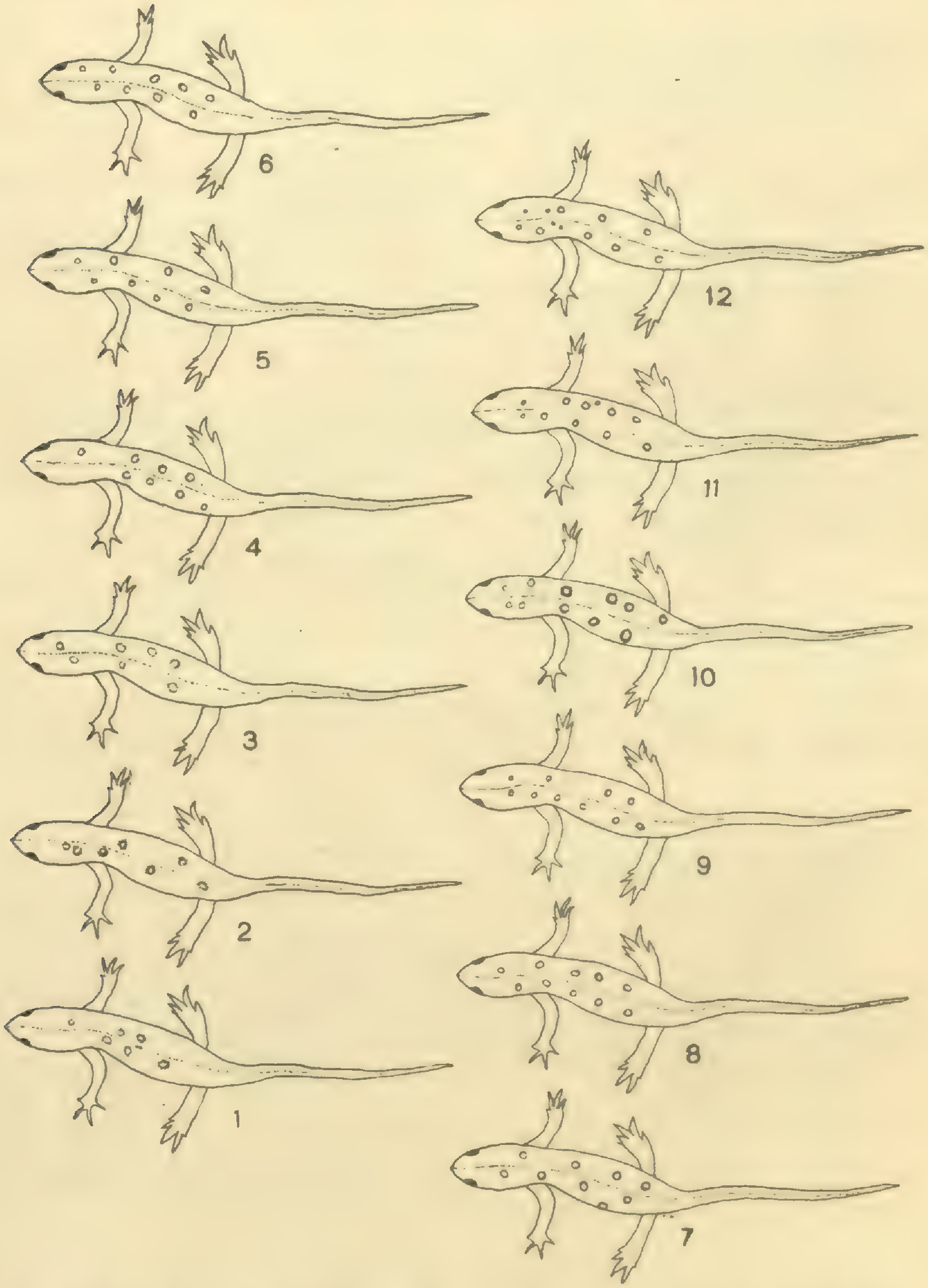
WHILE carrying on some experiments with the spotted newt, *Diemyctylus viridescens*, I was struck with the variation in the size, number and arrangement of the black-bordered vermilion spots so characteristic of this beautiful little salamander.

It is now generally recognized that this species exhibits two phases which were formerly described as distinct varieties or even species. As described by Gage[1] the young animal, which is terrestrial in habits, is red in color and was formerly called *D. miniatus;* later it becomes aquatic and its ground-color becomes olivaceous—permanently so, according to Gage. Against this dark ground-color (which is subject to considerable variation under different conditions even in the same individual) the bright red spots with their black borders stand out very strikingly.

It was with the olivaceous phase that I was experimenting, and it is upon this phase that the following observations are based.

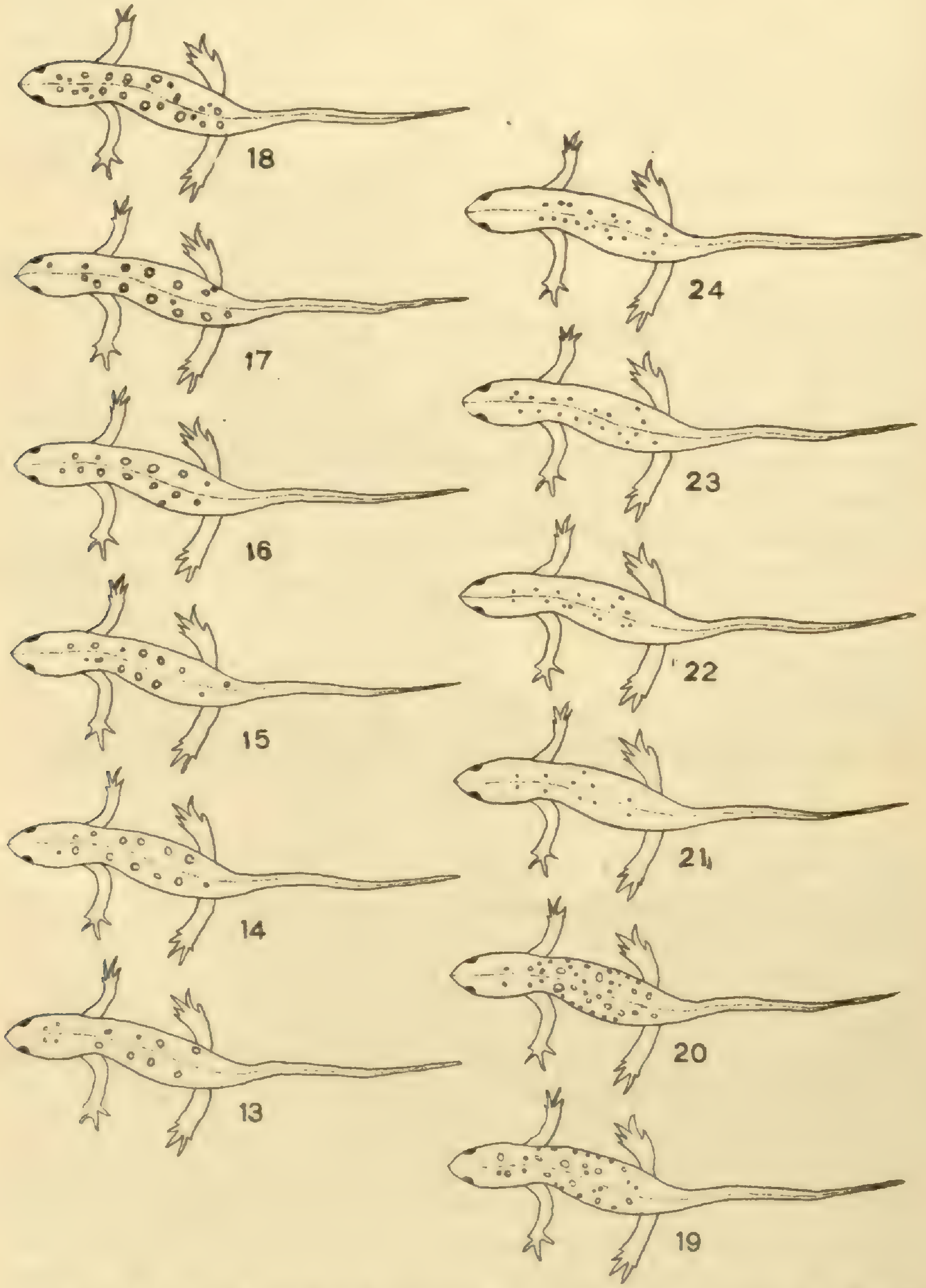
[1] Gage, S. H., "The Life-History of the Vermilion-Spotted Newt," AMER. NAT., December, 1891, pp. 1084–1103.

All the drawings were made from preserved material in which the vermilion spots had mostly faded to a white or pale pink color.



The first twenty figures were made from about three dozen specimens, probably all from the neighborhood of Morgantown. The last four figures are from animals that had been obtained from the Marine Biological Supply Company, Woods Hole, Mass.,

and had died, from time to time, in the laboratory aquaria. The mid-dorsal ridge is indicated in the figures by the dotted line.



Only the black-bordered vermilion spots were noted, the small black spots being too numerous and irregular to make it worth while to study them.

It will be noticed that in the animals from Woods Hole, shown in figures 21 to 24, the red spots were much smaller than most of

those on the animals from Morgantown. This was true of nearly but not quite all of the animals obtained from the north.

Cope says:[2]

> On each side of the vertebral line is a row of from three to six small round red spots, each with a black border. The rest of the surface is marked with small black points, which are smaller but more distinct on the lower surface.

Among all of the animals examined no two were spotted alike. They were sorted into groups according to the total number of red spots. The smallest number of red spots found was six; they were all of large size and arranged as shown in Fig. 1; only one animal with this number of spots was found.

Four animals were found that had seven red spots; Figs. 2 and 3 show the arrangement of the spots on two of these animals; all of the spots were large and of about the same size.

Four animals exhibited eight red spots, mostly large and of uniform size; two arrangements are shown in Figs. 4 and 5.

Three animals had nine red spots each, mostly large and of uniform size; Figs. 6 and 7 show two arrangements of these spots.

Seven animals had ten red spots each, this being the largest number of animals found in any group. The spots were mostly large and uniform in size; two arrangements are shown in Figs. 8 and 9. It will be noticed that in Fig. 8 the spots are arranged in fairly regular pairs.

Five animals had eleven red spots of somewhat more variable size than in the preceding. Figs. 10 and 11 show two arrangements of these spots; and Fig. 10, especially, shows wide variations in the size of the spots.

Three animals exhibited twelve red spots of variable size, two arrangements of which are shown in Figs. 12 and 13.

Two animals, shown in Figs. 14 and 15, exhibited thirteen red spots of various sizes.

Two animals had fourteen red spots; one of these animals is shown in Fig. 16.

Figs. 17, 18, 19 and 20 show the arrangements of red spots on four animals that had 15, 24, 29 and 39 spots, respectively. It will be noticed in these animals, especially in the last, that the large number of red spots is due to an increase in the number of very small spots, the number of large red spots being no greater than in the earlier individuals. Thirty-nine was the largest num-

[2] Cope, E. D., "The Batrachia of North America," *Bull. U. S. Nat. Mus.*, No. 34, 1889, p. 210.

ber of red spots found on any single animal. Only one animal in each of these last five groups was found.

Figs. 21 to 24, as noted above, represent animals obtained from Woods Hole; they have 11, 16, 19 and 20 spots, respectively, and it will be noted that all of the spots are small and of fairly uniform size.

CONCLUSION

It would seem from this hurried survey that the number, size and arrangement of the vermilion spots, so characteristic of *D. viridescens*, are quite variable, probably two animals very seldom being even approximately alike.

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WEST VIRGINIA UNIVERSITY,
MORGANTOWN

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THE
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NEW LIGHT ON BLENDING AND MENDELIAN INHERITANCE

PROFESSOR W. E. CASTLE
BUSSEY INSTITUTION, HARVARD UNIVERSITY

THE question whether blending or intermediate inheritance involves Mendelian principles is of prime interest to every student of genetics. The further question whether Mendelian characters are constant or variable is not less important. New light is shed on both these questions by a recent and valuable investigation by Y. Hoshino, "On the Inheritance of the Flowering Time in Peas and Rice."

The investigation is to be most highly commended for its thoroughness. It has involved the raising of over 30,000 plants extending over a period of 8 years. F_2 and F_3 generations have been raised three times and F_4 twice. The publication contains a careful summary and analysis of the facts observed. Above all it contains *facts* with a minimum of speculation, not facts so marshalled as to prove a particular theory and otherwise useless, but facts which the reader may study from any angle he chooses. I propose to exercise the privilege which our author's commendable method of publication makes possible, of utilizing his facts for testing a slightly different theoretical interpretation from that which he adopts, for I am acquainted with no material equally valuable in relation to the two questions stated above, though I have been engaged for many years in studying these same questions.

Hoshino crossed two varieties of peas which differed in

flowering time by about three weeks. The early variety was white-flowered, the late variety red-flowered. The F_1 hybrids were only a little earlier than the late variety in time of flowering and of course had red flowers, this being a dominant character. The F_2 hybrids varied in time of flowering throughout the range of both parent varieties and all intermediate points, but not beyond the range of the parents. Hoshino divides the F_2 plants into two main groups, an early flowering and a late flowering group, the two being separated naturally by a class of "minimal frequency" usually recognizable. A majority of the plants in the early flowering group had white flowers, a majority in the late flowering group had red flowers. This result shows that early and late flowering have a tendency to segregate from each other as Mendelian allelomorphs and that time of flowering is *coupled* with flower color. This fact of coupling, first observed by Lock, alone would suffice to show that time of flowering is inherited as a Mendelian character. The fact that the F_2 individuals apparently fall into two main groups, not into four or eight, indicates that one important Mendelian factor is concerned, not two or three. But Hoshino found that in F_3 four (not two) groups of relative constancy were obtained and this has led him to suppose that the inheritance of time of flowering depends upon *two* independent Mendelian factors, one of which (A, a) determines late or early flowering, while the second (B, b) supplements or modifies the action of A. He regards A as the *principal* factor, with which alone flower color shows coupling. The action of B is regarded as subsidiary. A characterizes late-flowering plants; a, early-flowering plants. B makes the flowering time later than it would be otherwise; b of course makes it earlier. The four supposedly homozygous sorts which are recognized are of the following formulæ:

aabb, early; aaBB, intermediate early; AAbb, intermediate late; AABB, late. Since red color of flowers is by hypothesis coupled with A, red flowers will predominate in the last two groups, white flowers in the first two

as observed, and the dividing line between them will correspond with Hoshino's class of minimal frequency in F_2. But the plants which Hoshino classified, on the basis of F_3 and F_4 tests, as extracted early (aabb) and extracted late (AABB) are usually not quite so early or late, respectively, as the uncrossed races. Hence he assumes the occurrence of "gametic contamination," and recognizes classes of "would be" or "pseudo"-early and "pseudo"-late. He also notes the occurrence of "qualitative" variation within the groups classed as "constant" early intermediate and "constant" late intermediate. That is one family supposed to be of the same genetic formula as another may throughout its entire range produce plants slightly *earlier* or *later* than those of the other family. This behavior is not ascribed to any difference in genetic formula, but to a slightly different value of the same gene in the two families.

The late race employed was also found to vary in lateness, one "pure line" derived from it being later than another. Crosses made with these two lines are reported separately. No factorial difference is recognized between them. Each is AABB, but one flowers a little later than the other and transmits this property to its descendants. Thus "qualitative" variation of a gene, *i. e.*, variation in its potency, is recognized by Hoshino. Aside from the occurrence of two pure lines in the late race, Hoshino considers "the flowering time quite fixed and unchangeable in the parent varieties," and cites his Tables IV–VI in support of this idea. Table VI is of particular interest in this connection because in this case seeds were planted of the earliest flowering and latest flowering individuals of the same pure line and descended from the *same individual grandparent*. These plantings constitute a test of the existence of genetic variation within the pure line. The progenies of the same grandparent plant (but of different parents) are so obviously alike and so little variable in flowering time that Hoshino has not considered it necessary to calculate their mean flowering time. But if this is done it affords unmistakable evidence that genetic varia-

tion occurs within these "pure lines" (see Table I). For in all but the last two of the thirteen cases tested the earlier parent has the earlier progeny. From long experience in studies of rats with such small differences as are here indicated I have no hesitation in concluding that fluctuating variation of genetic significance is here in evidence.

To recapitulate, as regards genetic variation in flowering time, Hoshino (1) recognizes that gametic contamination results from crossing early and late flowering varieties; (2) recognizes also that variation may occur among the cross-bred families, as well as in different pure lines of the uncrossed races, as regards the "quality," value, or potency of the same gene. (3) Although Hoshino does not refer to the fact, his observations show clearly that genetic variation of a graded or fluctuating sort occurs in at least one of the varieties which he crossed. It is probable that those varieties were as pure as are obtainable, but almost certain that their flowering time fluctuates slightly from genetic causes.

What I want to suggest is that in these several agencies we have a sufficient explanation of the variations observed in Hoshino's F_2, F_3, and F_4 generations, without invoking a two-factor hypothesis, one factor being enough. Hoshino has shown that a three-factor, or multi-factor hypothesis will not fit the facts observed. Will not *one* factor fit them quite as well as two, provided gametic contamination occurs, which he admits? The "pseudo-early" and "pseudo-late" classes Hoshino explains plausibly as due to gametic contamination. Could not the "intermediate early" and "intermediate late" be reasonbly explained as due to *further* contamination? For they intergrade with the pseudo-early and pseudo-late classes, respectively, and also with each other. From Hoshino's carefully controlled results, it is perfectly clear that early and late flowering are allelomorphs, and that segregation of early and late types occurs in F_2 but attended by gametic contamination. It is perfectly clear that the contamination is not uniform in amount. Some-

times little or no contamination is observable; sometimes it is considerable.

TABLE I

COMPARISON OF THE MEANS OF FAMILIES DESCENDED FROM THE SAME PURE-LINE GRANDPARENT, BUT FROM PARENTS OF UNLIKE CHARACTER
Based on Hoshino's Table 6.

Designation of Grandparent	Mean, Progeny of Earlier Parent	Mean, Progeny of Later Parent	Difference
Gp. I.4	59.96	60.11	.15
Gp. XVI.3	59.94	60.65	.71
Gp. XII.8	60.16	60.50	.34
Gp. VIII.3	63.65	63.98	.33
Gp. VIII.4	63.86	64.21	.35
C.12	60.25	60.58	.33
C. 65	59.43	60.05	.62
C. 61	62.91	64.18	1.27
C. 88	64.23	64.56	.33
Gp. XVI.3.5	66.45	66.63	.18
C. 65.27	66.22	67.30	1.08
Gp. VIII.4.23	71.75	71.20	–.55
C. 61.21	72.00	71.25	–.75

Contamination sufficiently great would account for the intermediate early as a *modified* early class and the intermediate late as a *modified* late class. The matter of coupling is unaffected by this hypothesis, since coupling is shown with only *one* factor, Hoshino's factor A. The observed variability and intergradation of the two intermediate classes favors a hypothesis of contamination rather than one of an independent modifying factor.

If we suppose modification due to gametic contamination to occur in half of the gametes formed by F_1 individuals and that this contamination is definite in amount (say equivalent to 5 days) the F_2 expectation would be exactly the same as from a two-factor system such as Hoshino adopts. As a matter of fact neither supposition is exactly correct. If we adopt contamination as a sufficient explanatory hypothesis, we must suppose the amount of contamination to be variable; if we adopt a definite modifying factor, we must suppose the amount of modification to be variable.

To make the matter clear, let us suppose the early race to be stable at 35 days between sprouting and flowering

and the late race to be stable at 55 days. According to Hoshino's hypothesis aabb stands for a 35-day period, and AABB for a 55-day period. If we assign to the assumed modifying factor B a delaying effect of five days, then the class aaBB (early intermediate) will have a value of 40 days, and the other homozygous class AAbb (late intermediate) will have a value of 50 days. Heterozygous classes will be intermediate as follows:

TABLE II

COMPOSITION OF F_2 ON HOSHINO'S TWO-FACTOR HYPOTHESIS, WITH EQUIVALENTS IN DAYS FROM SPROUTING TO FLOWERING

Designation and Expected Frequency	Homozygous	Heterozygous	
		Monohybrid	Dihybrid
1 aabb	35		
2 aaBb		37.5	
1 aaBB	40		
2 Aabb		42.5	
4 AaBb			45
2 AaBB		47.5	
1 AAbb	50		
2 AABb		52.5	
1 AABB	55		

If, however, we replace the modifying factor B in this scheme by a modification in A amounting to 5 days, then we can dispense with B and yet obtain exactly the same classes and in the same numerical proportions and with nearly the same expectations as regards their breeding capacity. Let us assume that a stands for a 35-day period, A for a 55-day period, and that modified a, which we will call a′, stands for a 40-day period, and modified A, which we will call A′, stands for a 50-day period and that all are allelomorphs of each other. Then the F_1 gametes will be a + a′ + A′ + A and F_2 will contain the classes shown in Table III.

It is evident that both schemes fit the observed facts fairly well. Either one will explain the decreased variability of F_3 as compared with F_2 and the production of several different types of F_3 families differing in the amount of their variability, some of which are relatively constant. But the former scheme will not answer with-

out the further assumption of gametic contamination (which Hoshino makes) and the latter must invoke contamination of different degrees in order to explain the pseudo-early and pseudo-late classes. The former scheme involves two explanatory principles, the latter only one. Other things being equal, the simpler hypothesis is to be preferred.

TABLE III

COMPOSITION OF F_2 ON A ONE-FACTOR HYPOTHESIS WITH CONTAMINATION OF A DEFINITE AMOUNT (5 DAYS) IN HALF THE GAMETES

Designation and Frequency	Homozygous	Heterozygous, All Monohybrid			
		Difference, Conj. Gam., 5	Difference 10	Difference 15	Difference 20
1 aa.........	35				
2 aa′........		37.5 (35:40)			
1 a′a′........	40				
2 aA′........				42.5 (35:50)	
2 aA........					45 (35:55)
2 a′A′.......			45 (40:50)		
2 a′A........				47.5 (40:55)	
1 A′A′.......	50				
2 A′A.......		52.5 (50:55)			
1 AA........	55				

Experiments decisive between the two hypotheses are difficult to devise, but certain tests are possible. On the hypothesis of Hoshino one would not expect to obtain a class splitting into homozygous early intermediate and homozygous late intermediate. On the alternative hypothesis such a class (40–50) should be obtainable. *A "constant" class exactly intermediate between the parent varieties* (say 45) *would be impossible on Hoshino's hypothesis,* unless he is willing to admit an indefinite amount of contamination, which, however, would render the two-factor hypothesis superfluous. On the alternative hypothesis such a constant intermediate class should be obtainable after a sufficient number of inbred generations. In reality Hoshino's observations show that *it is obtained in F_4 and is then more abundant than any other "constant" class.* The largest group of hybrid offspring in Hoshino's experiments and that belonging to the latest inbred generation is his F_4 generation raised in 1914 (see

his Tables G–U). This includes 231 families classed by Hoshino as "constant." They are descended from 15 different F_2 individuals ranging in flowering time (in 1912) from 48 to 65 days, this being practically the entire F_2 range. The combined range of the F_3 families raised in 1913 extended from 45 to 71 days. From certain individuals, selected at intervals throughout the ranges of the fifteen F_3 families so as to represent their complete variability seeds were planted which produced the F_4 generation. 421 such F_4 families were reared and studied and of these Hoshino regards 231 as "constant" because of the limited range of variation in flowering time of each. The others are regarded as still heterozygous. The mean flowering time of each of the "constant" families has been calculated by Hoshino and these means have the distribution shown in Table IV (omitting fractions from the class magnitudes which would make them .5 greater than those given in the table, but would not affect their distribution). It should be noted that on account of the peculiar weather conditions of 1914, the flowering time came about 10 days earlier than in the two previous years, the range of the F_4 means extending from 34 to 59 days, whereas the F_3 range of flowering time was from 45 to 71 days. Since both upper and lower limits of the range are displaced by like amounts and in the same direction, the general character of the distribution is not affected thereby.

TABLE IV

CLASSIFICATION OF MEANS OF F_4 "CONSTANT" FAMILIES FROM HOSHINO'S TABLES G–U

Classes	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59
White..	1	13	5	2		3	18	12	15	14	13	12	3													
Red or mixed		2	1	1		1	2	1	4	13	17	6	6	2		5	3	3	2	11	16	3	10	8	1	1
Totals.	1	15	6	3		4	20	13	19	27	30	18	9	2		5	3	3	2	11	16	3	10	8	1	1

Table IV shows unmistakably that the F_4 constant families fall into three natural groups, not four as Hoshino's hypothesis would lead one to expect. The

modes of these three groups fall at 35, 44 and 54, respectively, with frequencies of 15, 30 and 16, respectively. Each group is separated from the next adjacent by a gap (a class of 0 frequency). The modes of the early and late groups correspond closely with those of the uncrossed early and late parent varieties, which in this season had modes at 35 and 56, respectively. The third and largest group has its mode almost exactly midway between the modes of the early and late groups, with 8 intervening classes below it and 9 above. It would be difficult to imagine a finer example of a stable intermediate class produced by hybridization. For it will be remembered that every part of this population is stable, since it includes only families shown by Hoshino's breeding tests to be reasonably constant, those which he actually pronounced "constant." From it, therefore, one would need only to choose families of the desired flowering time, in order to have a complete succession of varieties from very early to very late.

But it may be asked, is the middle group possibly an "early intermediate" group of Hoshino's formula aaBB separated from the later groups by a class of minimal (0) frequency, as in the F_2 distribution? If so it should contain very few red-flowering families, no more indeed than the early group itself, since each would obtain red-flowered families only by cross-overs. Inspection of Table IV shows that this hypothesis is untenable. The truly hybrid origin of the middle group is shown by the large number of red-flowered or mixed families which it contains. Nine out of 12 of the F_3 families which contributed to the production of the middle group contained red-flowered or mixed red and white families. The middle F_4 group itself contained 90 white-flowered to 52 red-flowered or mixed families, whereas the early group contained 21 white: 4 red, and the late group contained only red-flowered or mixed families.

Hoshino observed that the flowering time of F_1 plants was close to that of the late parent, being only 2 or 3 days

earlier, and considers this to be evidence of dominance, but I am inclined to think it should be interpreted differently, for F_2 plants having the genetic properties of F_1 plants are in some cases at least (Hoshino's Tables T and V) much earlier in flowering time, being in fact almost exactly intermediate between the parent races, although in one family (S) the F_2 plant was late like F_1, but its F_3 and F_4 descendants covered the entire range, as did those of T and U. I am inclined to interpret the inheritance as truly *intermediate* and to explain the lateness of F_1 and of an occasional F_2 individual as due to physiological non-genetic causes. Recent observations made on size inheritance in guinea-pigs together with certain observations recorded by Hoshino lead me to this conclusion. When the small *Cavia cutleri* is crossed with the relatively large guinea-pig, the F_1 hybrids are larger than either parent, but the F_2 hybrids as a group are close to intermediate and only a little more variable than F_1. A stimulus due to crossing makes F_1 larger than its genetic constitution would otherwise make it, but the added size due to this stimulus does not persist to any great extent beyond F_1. Hoshino's F_1 peas probably possess a similar vigor due to crossing, which quickly disappears in later inbred generations. If this vigor due to hybridizations causes extra growth it may delay flowering time, for Hoshino, confirming Keeble and Pellew, has shown that late-flowering plants have longer internodes than early-flowering ones.

Hoshino, as we have noted, divides his F_2 families into two portions, early-flowering (chiefly white) and late-flowering (chiefly red). But we have seen reason to think that the F_4 families fall naturally into three portions, early, medium and late, and it is possible to divide the F_2 families in a similar way, though of course somewhat arbitrarily, classing as early those falling within the range of the early parent or a little beyond it, and as late those which fall within the range of the late parent, while those which lie between are placed in

the middle class. When the F_2 families are treated in this way we find the distribution of white-flowering and red-flowering plants shown in Table 5.

TABLE V

DISTRIBUTION OF WHITE- AND OF RED-FLOWERING AMONG THE F_2 PLANTS

Hoshino's Table	Early		Medium		Late		Total
	White	Red	White	Red	White	Red	
Table 2	49	13	20	114	7	168	371
Table 8, A_1	10	3	10	62	1	11	97
Table 8, A_2	17	2	2	20	3	25	69
Table 8, B_1	4	0	6	25	0	7	42
Table 8, B_2	17	0	7	37	5	30	96
Table 8, C	26	24	3	37	5	45	140
Total	123	42	48	295	21	286	815
Per cent. white	74		14		6.8		

Hoshino uses his F_2 tables as a basis for calculating the strength of the coupling between earliness and flower color, and concludes that the coupling is approximately 7:1. If, however, F_2 is divided as in Table 5, the coupling appears to be less strong, probably about as 4:1 or 5:1. The percentages of white-flowered plants expected in each group on various integral coupling ratios are as follows:

Coupling	Early Group	Medium Group	Late Group
4:1	64%	16 %	4 %
5:1	69%	14 %	2.8%
6:1	73%	12 %	2 %
7:1	76%	10.9%	1.5%
Observed	74%	14 %	6.8%

It will be observed that the percentage of white-flowered plants in the early group indicates about a 6:1 coupling ratio, but in the medium group, it indicates a 5:1 ratio, while in the late group it would indicate a 3:1 ratio. Much uncertainty exists as to the classification of many of the F_2 plants as regards flowering time, because of irregular and delayed germination. Undoubtedly the classes early, medium and late overlap, so that not much

reliance can be placed on the categories adopted. But the figures given indicate that Hoshino has estimated too high the coupling strength, and that more probably it does not exceed 5:1. This is not due to any inaccuracy in Hoshino's calculations, but would follow only if the hypothesis suggested in this paper is substituted for Hoshino's hypothesis.

If I have correctly interpreted Hoshino's observations, flowering time in peas is clearly a Mendelian unit-character, entirely devoid of dominance, so that a strictly intermediate hybrid form is the commonest end-product of a single cross between early and late varieties. Further, segregation is imperfect so that blending results, which becomes more and more complete with each generation of inbreeding. From the incompleteness of the blending in the F_1 zygote and so the imperfection of the segregation in the F_1 gametes, it follows that many different types of F_2 zygotes are produced, some of which are practically constant (homozygous) particularly those at either extreme of the series (the "early constant," "pseudo-early constant," "late constant" and "pseudo-late constant") and also at two intermediate points ("intermediate early" and "intermediate late").

Other F_2 zygotes, resulting from the union of gametes quite dissimilar, produce a highly variable F_3 progeny, but one which will give rise to F_4 families individually less variable for two reasons: (1) because the process of blending continues and so gametes produced by the same zygote become more like each other than were the parent gametes of that zygote, and (2) because heterozygotes under self-fertilization tend to produce about 50 per cent. of homozygous offspring, while homozygotes produce only homozygous offspring.

The entire population therefore will in accordance with recognized Mendelian principles gradually resolve itself into relatively constant self-fertilizing lines. But because of the slow but continuous blending which occurs, these pure lines will in a very few generations form a complete

gradation of forms connecting one parental mode with the other. The most numerous of these intermediate forms will in F_4 and later generations be that which is midway between the modes of the respective parents.

This case throws a flood of light on the nature of blending and of Mendelian inheritance and of their relations to each other. In typical Mendelian inheritance determiners of allelomorphic characters may meet each other generation after generation in a common zygote, separating again in gametogenesis without apparent modification of either in consequence of their conjugation in a heterozygote. This is well illustrated in the color inheritance of animals and plants.

In typical blending inheritance the determiners of contrasted parental conditions apparently blend into a determiner of intermediate character, the gametes formed by an F_1 individual being practically as uniform in character as those of either parent individual. Blending is illustrated in the inheritance of ordinary size differences in birds and mammals (Castle, 1916).

A third type of inheritance must now be recognized which is a compromise between these two, for it exhibits Mendelian segregation of the contrasted parental conditions but with modification due to partial blending of the unlike determiners in the F_1 zygote. The blending increases and evidences of segregation decrease with every generation during which the contrasted characters remain in conjugation. Consequently with every generation of inbreeding or self-fertilization following a cross of this sort, a stable intermediate class is more and more closely approached until its realization is complete. See Marshall (1916) on the Corriedale breed of sheep. The existence of this third type of inheritance was pointed out by Castle and Forbes (1906) in the case of hair-length in guinea-pigs and by Castle (1906) in the case of polydactylism in guinea-pigs. The opinion was then expressed that "more characters fall in this category than in any other." Hoshino's observations on flowering time in peas (if I

rightly interpret them) fully establish the existence of this third type of inheritance. Incidentally they indicate that the Nilsson-Ehle principle of multiple determiners to explain blending inheritance is quite superfluous.

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THE OCCURRENCE OF THREE RECOGNIZED COLOR MUTATIONS IN MICE

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THE common wild mouse, *Mus musculus*, has a type of coat-color pattern well known to geneticists as being characteristic of almost all wild species of rodents. This pattern is commonly called the "agouti" pattern. It consists in a "ticking" or "banding" of the hairs of both dorsal and ventral surfaces. On the dorsal surface each hair has a subapical band containing yellow pigment. The tip of the hair is black pigmented, while the half proximal to the yellow band is dark, containing both black and brown pigment granules. The general effect of the dorsal surface, when considered as a whole, is dull, brownish gray. The ventral surface is distinctly duller and paler than the dorsal. The distal half of the hair is lightly pigmented with black and occasionally with some yellow pigment, while the proximal half is much more heavily pigmented with black and brown granules. The general impression, conveyed by the ventral surface, is dull faded gray. This fact has led to the adoption by various investigators of the term "gray-bellied agouti" to describe the wild mouse color pattern.

In addition to this gray-bellied agouti another type of agouti house mouse has been reported as occurring wild in certain localities. This type has been used in genetic investigations by Cuénot and by Morgan.[1]

The chief difference between the gray-bellied agouti and the aberrant type is that the latter has, generally speaking, more yellow and brown and less black pigment. This is especially true of a band of hairs which runs laterally along

[1] Morgan, noticing that Cuénot's mice of this variety had a small patch of reddish brown hair between the front legs while his own mice had not, has suggested the possibility that the variety with which he worked was not identical with that described by Cuénot. I have at present animals of this color variety with and without the spot and therefore hope soon to have a definite answer to this question.

the boundary of the lighter ventral hairs between the front and hind legs. This streak is very reddish in color and adds to the lighter and warmer coloring of the dorsal surface. Ventrally, the tips of the hairs are to all extents and purposes unpigmented and this produces a white or nearly white ventral surface. The proximal half of the hair is dark pigmented, a distinction between this type of white area and that found in spotted animals where the hair is white throughout its length. This type of agouti has been given the name of white-bellied agouti (gris à ventre blanc; Cuénot).

In crosses with gray-bellied agouti the white-bellied form is epistatic. It can be obtained in a homozygous condition and is, as stated by Cuénot and by Morgan, one of a series of four allelomorphic forms of coat pigmentation.

It is the appearance of this epistatic "white-bellied" agouti in two experiments involving gray-bellied agouti and non-agouti mice that I wish to record.

Experiment *A*

In 1913, a cross was made between wild gray-bellied agouti mice and a race of dilute brown mice which I had started in the laboratory of the Bussey Institution.

The agouti race used was directly descended from wild animals without any out crossing with any domesticated mice. It therefore represented a stock of wild mice raised for several generations in captivity.

From this stock of agoutis I have recorded 531 young. All of them were gray-bellied agoutis[2] of the type that one might catch wild in any house. A certain amount of variation in intensity of pigmentation occurred among these mice. This variation, however, was not different so far as could be observed from that shown by a considerable number of specimens of this species from any single locality, judging from skins in the museum of comparative zoology of Harvard University. Among these skins, and

[2] The brown agoutis recorded later in this paper are of the dark or "gray-bellied" type.

among the mice raised from the gray-bellied agouti stock, there was not even an approach to the white-bellied agouti type.

The dilute brown animals which were used belonged to a race descended from a single pair of dilute brown mice which had been tested by suitable matings in order to establish their homozygosity. These animals in common with other "non-agouti" varieties of mice lack all visible traces of the agouti pattern and are with the exception of a slightly lighter ventral than dorsal surface uniformly pigmented throughout.

The cross between these two races, then, was one between a gray-bellied agouti and a "non-agouti" race. Both races used as parents were therefore color varieties which were hypostatic to the white-bellied agouti type and which for this reason could not carry that pattern as a recessive.

Four females of the dilute brown race were crossed with a gray-bellied agouti male 131 and produced 26 gray-bellied agouti young as shown in Table I.

TABLE I

GRAY-BELLIED AGOUTI

Mating	
♀ b1 × ♂ 131	6
♀ b2 × ♂ 131	8
♀ b3 × ♂ 131	6
♀ b4 × ♂ 131	6
	26

The F_1 gray-bellied agoutis were more intensely pigmented than their gray-bellied agouti parents. This deepening of color has frequently been observed in crossing wild agouti varieties with tame races and is probably due to modifying factors introduced by the particular tame race used or else to a general acceleration of pigment production due to heterozygosis.

The F_1 animals were crossed *inter se* and 285 F_2 young were raised. Of these 14 died at too early an age to have their color recorded.

In an earlier paper (1913), I have reported a cross similar to the one here recorded and have shown that three pairs of alternative mendelizing factors are involved. These are:

A—gray-bellied agouti.	a—non-agouti.
B—black pigment.	b—no black pigment.
D—intense pigmentation.	d—dilute pigmentation.

The gray-bellied agouti parent was homozygous for the factors for gray-bellied agouti, black and intensity being AABBDD in zygotic formula. The dilute brown parent was homozygous for the hypostatic conditions, non-agouti, no black pigment, and dilute pigmentation, and was, therefore, aabbdd in formula.

The F_1 gray-bellied agoutis would of course be heterozygous for all three pairs of factors and would be AaBbDd in formula. When such F_1 hybrids are crossed together, eight color classes of young are expected in a 27, 9, 9, 9, 3, 3, 3, 1 ratio. As will be seen from Table II, this result was approximated with the addition of a ninth and unexpected color variety, the white-bellied agouti.

TABLE II

Mating	F_2 Generation									
	Black Agouti Gray-belly	Black Agouti White-belly	Black	Brown Agouti	Dilute Black Agouti	Brown	Dilute Black	Dilute Brown Agouti	Dilute Brown	?
A 32×13	4	—	—	2	1	2	—	1	—	1
B 14×17	2	—	—	—	1	—	1	2	—	2
C 8×20	8	—	6	3	3	1	1	—	1	6
D 16×18	4	—	—	4	1	—	1	—	—	—
E 33×22	2	—	—	—	—	—	—	—	—	5
F 21×20	15	1	2	4	3	3	3	2	1	—
G 9×31	10	—	4	8	4	—	3	2	1	—
H 7×31	3	—	4	1	2	—	1	—	—	—
I 6×18	16	—	3	3	3	2	3	4	—	—
J 23×17	9	—	3	3	2	—	—	—	—	—
K 30×19	24	—	6	4	5	1	2	2	2	—
L 15×13	15	—	3	3	4	1	—	—	—	—
M 12×22	12	1	4	2	3	1	2	1	—	—
Obtained numbers	124	2	35	37	32	11	17	14	5	14
Expected on three factor basis	116.1	—	38.7	38.7	38.7	12.9	12.9	12.9	4.3	—

It will be noticed that the approximation to the expectancy on the basis of three pairs of Mendelian factors is extremely close. The occurrence of the two "white-bellied" agouti young is the matter of especial interest. These two young, one a male the other a female, are apparently identical with the gris à ventre blanc of Cuénot, judging from his description.

The question may quite naturally arise as to the possibility that the white-bellied agouti animals in F_2 had arisen by an accidental cross with some white-bellied agouti male in an adjoining cage. This possibility, however, is obviated by the fact that the F_2 generation white-bellied agoutis appeared in different cages at the Harvard Medical School where there had been *no other white-bellied agoutis* for more than a year before the appearance of the mutant animals. The two F_2 white-bellied agoutis must then be considered as true mutants epistatic to the type from which they originated.

Further evidence is provided by the F_3 generation, a tabulation of which is given below.

It will be observed that there is one "white-bellied" agouti among the 624 animals comprising the F_3 generation. This one mutant occurred as a descendant of a family of F_2 animals shown in Table II, mating J. This mating in F_2 gave 9 black agouti and 3 brown agouti young, none of which were "white-bellied" and the mutation therefore originated in a gamete formed by one of the F_2 generation animals while the other two white-bellied agoutis originated as mutations in the gametes of F_1 animals.

This recurrence of the mutation is a matter of considerable interest and indicates a germinal change, the occurrence of which is largely irregular, having some underlying cause or causes which become operative in animals of this particular hybrid race.

That hybridization between the same wild agouti and dilute brown races does not always produce this or any other recognizable mutation from the eight expected color

TABLE III

F_2 Parents	F_2 Mating	F3 Generation: Black Agouti Gray-belly	Black Agouti White-belly	Black	Brown Agouti	Dilute Black Agouti	Brown	Dilute Black	Dilute Brown Agouti	Dilute Brown	Pink-eyed Black Agouti	Pink-eyed Dilute Black
M	164 × 161.....	2	—	—	2	—	—	—	—	—	—	—
	163 × 161.....	3	—	3	—	—	1	—	—	—	—	—
	72 × 76......	—	—	—	—	20	—	14	4	—	—	—
	163–164 × 161.....	14	—	1	11	—	2	—	—	—	—	—
L	331 × 335.....	—	—	—	2	—	—	—	—	—	—	—
	167 × 168.....	10	—	10	5	2	2	—	1	—	—	—
	165 × 168.....	2	—	1	—	—	—	1	—	—	—	—
	89 × 86......	12	—	—	—	6	—	—	—	—	—	—
	84 × 86......	16	—	—	—	9	—	—	—	—	—	—
	84–89 × 86......	30	—	—	—	3	—	—	—	—	—	—
	165–167 × 168.....	7	—	5	2	2	1	—	1	—	—	—
K	280 × 320.....	1	—	1	—	—	—	—	—	—	—	—
	130 × 135.....	3	—	2	2	—	2	—	—	—	—	—
	57 × 56......	3	—	2	7	1	—	—	1	2	—	—
	55 × 56......	2	—	6	—	—	—	1	—	—	—	—
	51 × 56......	4	—	6	2	—	2	—	—	—	—	—
	51–57 × 56......	3	—	4	1	2	1	1	2	1	—	—
J	145 × 142.....	4	—	—	2	—	1	1	3	1	—	—
	144 × 142.....	3	1	1	—	—	—	—	1	—	—	—
	91 × 93......	10	—	17	—	2	1	3	—	—	—	—
	90 × 93......	—	—	35	—	—	8	6	—	5	—	—
	144–145 × 142.....	3	—	1	—	3	—	—	1	—	—	—
F	136 × 137.....	9	—	1	3	—	—	—	—	—	—	—
	136–140 × 137.....	6	—	1	2	—	1	—	—	—	—	—
G	185 × 182.....	—	—	—	42	—	10	—	2	—	—	—
H	110 × 106.....	3	—	5	—	3	3	5	2	—	—	—
	102–103 × 101–105.	3	—	1	—	—	—	4	—	—	3	2
I	172–174 × 171......	16	—	—	10	17	—	—	14	—	—	—
	80 × 78......	4	—	1	—	—	—	—	—	—	—	—
	79 × 78......	6	—	7	—	—	—	—	—	—	—	—
	79–80–82 × 78......	16	—	20	—	—	—	4	—	—	—	—
	79–80 × 78......	5	—	6	—	2	—	—	—	—	—	—
		200	1	137	93	72	35	40	32	9	3	2

varieties shown in Table II, is proved by the following experiments.

In 1911 a parallel cross was made between wild agouti and dilute brown races. The making of this cross I have recorded (1913; p. 55, Cross 20*a*; p. 52, Cross 10*a*, etc.) in a previous paper. The first hybrid generation consisted of gray-bellied black agouti animals, 8 in number. The second hybrid generation consisted of 55 animals which were of the expected color varieties. In the third hybrid and ensuing generations, including back crosses, I have recorded more than 4,500 young. None of these animals

were white-bellied agoutis or indeed of any but the expected eight color varieties. This proves conclusively that the hybridization of these two races does not necessarily cause the white-bellied mutation to appear.

The fact that the white-bellied mutation has occurred in wild races of *Mus musculus* as shown by the capture of white-bellied mice would indicate that this mutation does not *necessarily* depend for its origin upon the presence in the germ cell of any of the recognized color factors *in a heterozygous condition.*

The test by breeding of the white-bellied agoutis obtained in the F_2 and F_3 generations has been attempted. One of F_2 generation mutants has not yet been bred, but the other F_2 and F_3 white-bellied agoutis have shown that their color pattern was epistatic to gray-bellied agouti and non-agouti. In these respects, the mutants have behaved in a manner identical with the gris à ventre blanc mice of Cuénot or the white-bellied agoutis of Morgan.

The observed facts therefore prove clearly the origin of this epistatic white-bellied agouti mutation at three different times in the course of this experiment.

It is interesting to note that there has been no selection in the direction in which the mutation occurred as was the case in the experiments of Castle and Phillips (1914) on hooded rats.

Although the white-bellied agouti may easily be considered merely an increased state of activity of the agouti factor beyond the gray-bellied stage, it is certain that it arises, not as the product of a series of small gradual changes, but suddenly and distinctly, without warning, and that after its appearance it behaves at once as a mendelizing character.

Taking into consideration the facts now recorded concerning this particular mutation we can say that its origin is apparently not solely dependent upon any of the known genetic processes. Inbreeding, hybridization, selection, none of them is indispensable to the occurrence of the mutation. The underlying cause or causes are at present clearly outside the bounds of analysis.

EXPERIMENT *B*

In 1912 I started, on a small scale, an experiment which had for its object the modification of the agouti pattern by repeated crossing with black (non-agouti) animals. It was thought that if the gametes of agouti animals showed quantitative variation in the factor underlying the agouti pattern repeated crossing with non-agoutis would succeed in decreasing the amount of the agouti character. It follows that if this was accomplished by contamination between the agouti gamete and the non-agouti (black) gamete, the non-agouti animals should show increasing traces of the agouti character as the agouti animals showed a diminution of that character.

The method followed was to cross black agouti animals derived from a sooty yellow stock with blacks from the same stock and selecting the blackest agoutis from each litter repeat the process. All the agoutis used were thus heterozygous for the agouti factor. This process was repeated for more than seven generations during which approximately 400 young were recorded. From the outset two facts were evident. *First, the agoutis grew distinctly blacker: second, there was no corresponding sign of contamination on the part of the non-agouti animals, but they too grew blacker.* It appears, therefore, that there was no sign of modification of the *factor* underlying the agouti pattern; but that a race was isolated by selection which showed a distinct increase in depth of pigmentation. In this connection it is interesting to note that while the agouti animals grew blacker and the yellow areas in the hair decreased *in extent,* the yellow pigment itself was a deeper richer yellow than that of the ordinary black agoutis. The yellow pigment had been increased in *depth* while the black pigment was being increased in *depth* and in *extent,* two entirely distinct processes.

All the agoutis up to the third generation were gray-bellied agoutis usually having more dark pigment on the ventral surface than is found in wild mice. In the third generation there occurred an agouti with a distinctly yel-

lowish tinge to the under surface. The dorsal appearance of this animal was apparently the same as the other agoutis of this generation, a deep rich agouti with much black pigment. The ventral surface was, however, distinctly lighter in color than the other agoutis and showed a *decrease in dark pigment* as compared to them.

The yellow-bellied variation reappeared in the immediate progeny of the original yellow-bellied female and a race of these animals was established. When single mating tests were made to determine its behavior it *was found that when yellow-bellied agoutis one of whose parents was a non-agouti were crossed with non-agouti, only yellow-bellied agouti and non-agouti young resulted.* This fact indicated that yellow-bellied agouti fell somewhere in the series of multiple allelomorphs recorded by Cuénot and by Morgan.

At about this time English "black and tan" mice[3] crossed with white-bellied agoutis were found to give among their progeny agouti young almost identical with the yellow-bellied agoutis described above.

It at once suggested itself that the yellow-bellied agoutis occurring in my selection experiment were really *white-bellied agoutis* with one or more modifying factors which encouraged a higher degree of pigmentation than is normally found. This increase in an oxidation process would account for yellow pigment appearing in the tips of the ventral hairs which in ordinary white-bellied agoutis are unpigmented.

This supposition was further favored by the fact that as certain of the yellow-bellied agoutis grew to be old mice they showed a diminishing depth of pigmentation, *and developed typical white-bellied agouti coat color.* In old age they were not visibly different from some of the darkest white-bellied agoutis descended from a male of this variety kindly sent me by Professor Morgan.

[3] Black and tan mice are a very dark type of yellow. Only a small amount of yellow appears on the sides, while the ventral surface is a deep rich reddish yellow. Black animals descended from black and tans are coal black with deep black ears, feet and tail.

One breeding experiment is interesting in showing the probable identical nature of the factor underlying the yellow-bellied and white-bellied agouti types. Two yellow-bellied agouti females known to carry non-agouti were mated with a white-bellied agouti male known also to carry non-agouti. On the supposition that the white-bellied and yellow-bellied mutations are the same, this mating should produce two types of young, agouti (either yellow or white-bellied, not both) and non-agouti in a 3:1 ratio. If on the other hand yellow-bellied agouti depended on a distinct factor falling in the multiple allelomorph series hypostatic to white-bellied agouti, the mating should produce three types of young, white-bellied agouti, yellow-bellied agouti, and non-agouti in a 2:1:1 ratio. Finally, if *yellow-bellied* agouti depended upon an *independent modifying factor which was acting upon a gray-bellied agouti animal,* one of two results should be obtained. If the factor was epistatic we should have white-bellied agoutis (plus the modifier), yellow-bellied agoutis and non-agoutis in a 2:1:1 ratio. If the modifier was hypostatic in the cross, white-bellied agouti, gray-bellied agouti and non-agouti young should occur in a 2:1:1 ratio. The three litters obtained have totaled 19 young; 11 white-bellied agouti and 8 non-agouti. The two classes of young seem to indicate strongly the correctness of the view that yellow-bellied agoutis are in reality white-bellied agoutis plus a darkening modifying factor or group of darkening modifiers the nature of which is not yet sufficiently clear to allow further description.

The point of extreme interest is that the white-bellied agouti mutation has occurred in a race which was being selected in directly the opposite direction from that taken by the variation. White-bellied agoutis represent a *stronger* agouti factor than the gray-bellied agouti factor, while every effort was being made in this experiment to weaken the gray-bellied agouti factor. It appears that the occurrence of the white-bellied mutation in experiment *A,* above recorded where *no selection* was being

exercised, and the independent occurrence of the same mutation in experiment *B against* the course of selection are evidences that *the direction of mutation is largely if not entirely independent of selection and that the occurrence of the plus mutant rat in the plus selection series of Castle and Phillips before referred to, is in all probability a matter of coincidence rather than the result of selection as they have hinted.*

Pink-eyed Mutation

It will be noticed in the table showing the F_3 generation of the previous experiment (*A*) that among the 624 young recorded, 5 are pink-eyed. The pink-eyed mutation is hypostatic to dark-eye color and has been known for some time (see especially Castle and Little, 1909, Durham, 1911). These young all appeared in a single pen in which were two females and two males all of a single litter. Eight dark-eyed and five pink-eyed young were produced by these mice.

Up to the appearance of the pink-eyed young it was not suspected that the pink-eye factor in any way entered into the experiment. It was certain that one parent stock consisted of pure wild mice and that the pink-eye factor, which is a recessive, was not brought into the cross by this parent.

The pink-eye mutation, being recessive, could easily have been latent in the cross for some time without the combination of gametes necessary for its manifestation in a zygote having been realized. The dilute brown animals from which the second parent race was descended had originally been tested by breeding and had been found to be entirely free from the recessive pink-eye factor. Since, however, this mutation *appeared in only one F_3 family* it seemed distinctly unlikely that the original wild male, 131, which had been used as the male parent of all families, was the animal through whose gametes the mutation came into the cross. On the other hand, as there were four dilute brown females (Nos. b1, b2, b3, b4,) used in

producing the F_1 generation, it seemed quite likely that one of these first introduced the pink-eye factor.

If such was the case the animal in question would be female No. b2. This mouse was the dilute brown ancestor of the particular group of F_2 animals which in turn were the parents of the pink-eyed mutant individuals.

The suggestion that the dilute brown parent is the animal which introduced the pink-eye factor is supported by the fact that *at about the same time at which the mutation appeared in the F_3 generation of this experiment it also appeared within the "pure" dilute brown race.* This makes it extremely probable that the mutation had already occurred within the dilute brown race and was brought into the cross by a single dilute brown female which because of the fact that it was dark-eyed concealed the presence of the recessive pink-eyed factor which in all probability existed in approximately half its gametes.

Unfortunately at the time that this mutation appeared in F_3 the dilute brown great grandparent had died. A breeding test was therefore impossible in order to ascertain whether she actually carried the pink-eye factor in one half of her gametes.

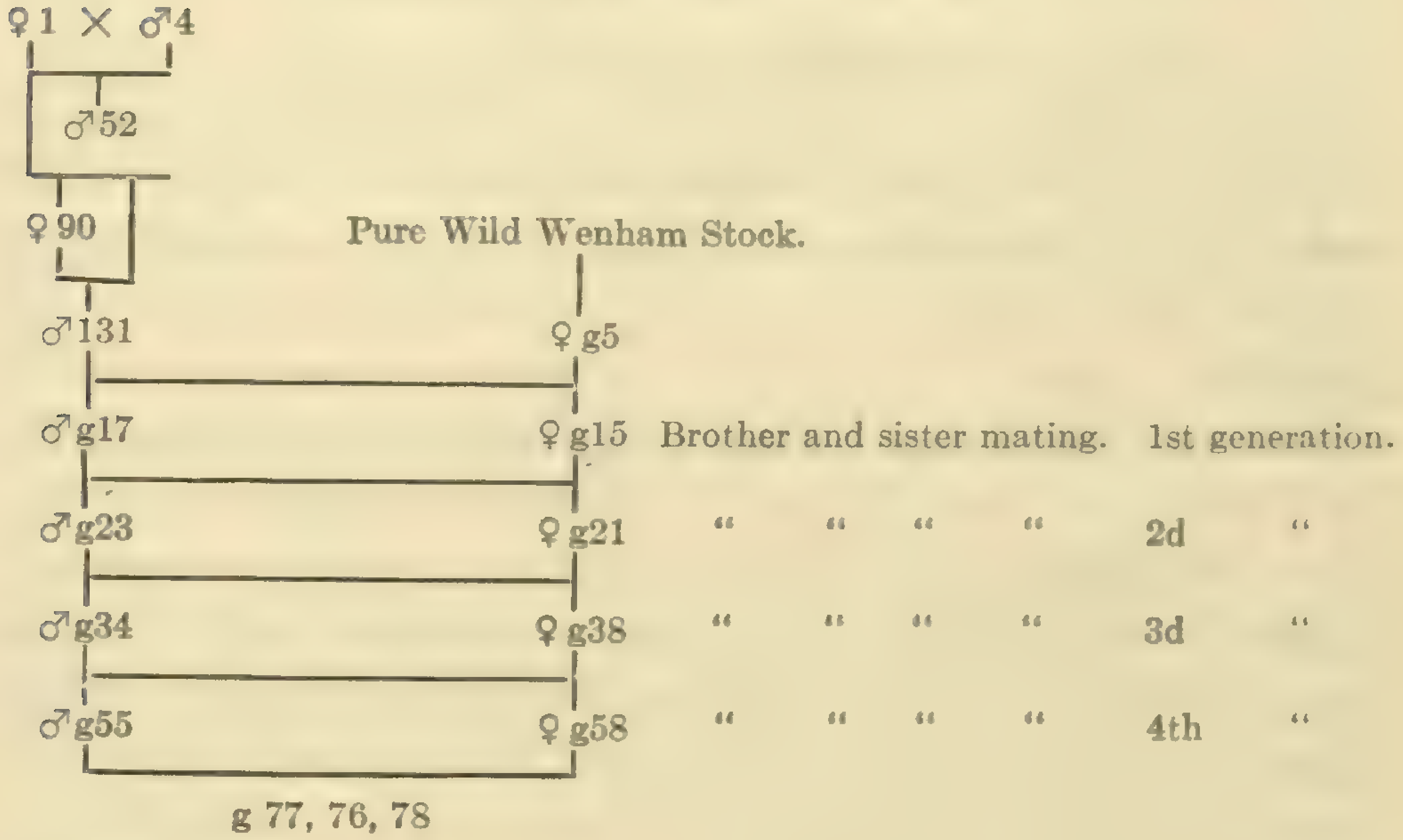
EXPERIMENT *C*

Another mutation, this time of the black producing factor, has occurred in a stock of pure wild mice, the original individuals of which were caught either in Wenham, Mass., or in Forest Hills, Mass., in 1912.

The particular family in which this mutation occurred is shown in Table IV. As will be seen, two wild mice both caught at Forest Hills, Mass., were bred together, these are female 1 and male 4. They and all their descendants, unless especial mention of the fact is made, will be considered black agouti in color. That is to say they were in appearance ordinary wild house mice. Female 1 and male 4 gave among their progeny male 52, who was crossed back to his mother and thus gave rise to female 90. She in turn was crossed back to her father, male 52, and from this mating male 131 was obtained.

TABLE IV

ANCESTRY OF BROWN-AGOUTI MUTANTS



8 Black agouti.
3 Brown Agouti.

Male 131 was used in many crosses, one of which was with female g5. This female was a pure wild mouse taken from a stock pen of wild mice which for several generations had been reared in captivity by Dr. J. C. Phillips at Wenham, Mass. From this mating came 6 black agouti young in a single litter. Two of these young, female g15 and male g17, brother and sister, were mated together and produced 11 black agouti young, two of which, male g23 and female g21, were mated together. This brother and sister mating gave 16 black agouti young from which another brother and sister mating, male g34 and female g38, was made. From this pair was obtained 13 black agouti young, among which were male g55 and female g58, the parents of the mutants. These two mice male g55 and female g58 have had three litters of young. The first born in December, 1915, consisted of three animals, male g77 *Brown Agouti,* female g78 *Brown Agouti* and female g76 *Black Agouti.* The appearance of the brown agoutis was entirely unexpected and was thought to be possibly due to an error in records. A sec-

ond litter however, born in February 1916 after the parents had been under careful observation, consisted of two black agoutis, a male and a female, and *one brown agouti a female.* The third litter of five is all black agouti.

This is a clear case of mutation within a closely inbred race, and is interesting to contrast with Experiment *A* already referred to, in which a mutation occurred in hybrids.

There is one fact of possible interest in connection with the mutations recorded in Experiment *A* and in this experiment. Male 131, black agouti, is a common ancestor of all the races in which the mutations occurred. It has been shown that the evidence is *against* his having introduced the pink-eyed mutation and that this probably came from the dilute brown race.

For the other two mutations, however, the white-bellied agouti and the brown agouti types, it is theoretically possible that male 131 possessed or transmitted an instability of germplasm which has manifested itself in the cropping out of these mutations among his descendants. Fortunately the stock within which the brown agouti mutation arose is being carried on in single pair, brother and sister, matings. By this method we should be able to recognize mutations at the earliest possible moment after their occurrence.

Summary

To sum up the facts above recorded it may be stated that:

1. A previously recorded mutation of the gray-bellied agouti pattern, known as white-bellied agouti, has arisen in two experiments on color inheritance in mice.

2. In experiment *A* it has arisen independently three times in a hybrid race of mice.

3. In this experiment there has been no selection in the direction of the mutation.

4. In experiment *B* it has arisen once in an inbred race in which selection was being carried on.

5. In this race the mutation represents a variation in

exactly the opposite direction from that in which the selection was being made.

6. A recessive pink-eyed mutation has occurred in a closely inbred dilute brown race. A similar mutation has appeared in a hybrid race into which one animal from the dilute brown race probably introduced the mutation.

7. A mutation involving loss or suppression of the black producing factor has arisen in a stock of inbred wild mice. This has caused the appearance of brown agouti young.

8. The wild race in which this occurred is related to the hybrid race (see conclusion 2) in which the white-bellied agouti mutation appeared three times. The suggestion is offered that a tendency to germinal instability may have been transmitted by male 131 a common ancestor of both races.

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THE MECHANISM OF CROSSING-OVER III

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V. An Experiment to Determine the Linkage of Many Factors Simultaneously

A more exact knowledge of the interference of one crossing-over with another required an experiment, or series of experiments, in which the distance between the two points of crossing-over in cases of double crossing-over could be more accurately determined. In an experiment involving only three factors—A, D and H—if a double cross-over occurs, all that can be known is that crossing-over has occurred at the same time somewhere between A and D, and somewhere between D and H, but nothing can be known of the precise location and distance apart of the two points of crossing-over, except that they could not be further apart than A and H. On the other hand, if the inheritance of four points could be followed—say, A, D, F and H—then the distance between the two points of crossing-over could be determined a little more exactly, for a double crossing-over involving breaks between A and D, and between D and F, would cut out a shorter segment of the chromosome than one occurring in regions A–D and F–H. And the more numerous were the factors that could be followed—other things being equal—the more exact the determination would become. At the same time, it might be possible by comparing the results of a *series* of different experiments to arrive at the desired end with the three-factor method also. For example, the difference in frequency between the double cross-overs obtained in an experiment involving A, B, C and in an experiment involving A, B, D, must obviously be due to the double cross-overs involving regions A–B and C–D,[1] except in so far as these differences are due to the random deviation of different samples from each

[1] For region BD is made up of BC + CD. Therefore double crossovers involving AB and BD really consist of double cross-overs involving AB and BC plus those involving AB and CD. Consequently, if we subtract the number of double cross-overs involving AB and BC from the number involving AB and BD, we obtain the number involving AB and CD.

other, or to actual differences in the behavior of the chromosomes in the two experiments. As the last two influences seemed by no means negligible, and as the experiment involving many points at once gave a more direct and graphic picture of the results, it was decided to use this method of attack in preference. Moreover such an experiment incidentally afforded an opportunity of attacking certain other questions, such as the effect, on crossing-over, of having the two chromosomes different in regard to many factors. Meanwhile, the indirect method of attack would be followed by other workers, and the two sets of results could finally be used as checks upon each other.

The many factor method is in itself for several reasons very laborious, but this is compensated for by the fact that when the results are obtained they are the equivalent of an entire series of different experiments involving in turn the linkage of each factor with every other one, and indeed, the results are much more than the equivalent of these, for in the latter cases the linkages are obtained in different experiments, so that there is much more chance for error in determining the relation of one linkage to another.

It was evident from the outset, however, that there was one very important obstacle to be overcome in any study of linkage exact enough to give useful information regarding coincidence, and that the difficulty was especially great in the type of experiment contemplated. The difficulty referred to is "differential viability," for it is found that in nearly all experiments not involving the characteristics of seeds or other structures dependent upon the maternal organism for support, the individuals belonging to different genetic classes may be very differently equipped in respect to their ability to meet the struggle for existence. Thus, since the count generally takes note only of the individuals which survive. the ratios obtained may be very different from the ratios of the different classes of gametes. These discrepancies apply especially to forms like flies, the larval life of which can not be well controlled, and they are, of course, particularly great in crosses involving many factors at once.

Before considering the means by which differential viability may be reduced in crosses of multiple stocks, it may not be out of place to explain two methods I devised for getting a more correct estimate of the gametic ratio in back-crosses involving only two pairs of linked factors.

Let us say that the gametic ratio is r(AB):r(ab):s(Ab):s(aB). Assume that when A is present the viability of the flies is reduced so that only A′ per cent. of those which would otherwise survive, now come to maturity, and assume that factor B lessens the output to B′ per cent. of what it otherwise would be; similarly a and b, when present, lower the output to a′ per cent. and b′ per cent., respectively. Then the relative number of AB individuals which survive will be rA′B′ (per cent. marks are omitted for brevity); the relative number of Ab will be sA′b′, etc. The actual, observed, numbers will be some multiple (k) of these relative numbers; thus the number of AB individuals actually found will be krA′B′, the actual number of Ab will be ksA′b′, etc. It can now be shown that the true gametic ratio (r:s), which it was desired to find, may be derived by the formula

$$\sqrt{\frac{AB \times ab}{Ab \times Ab}}$$

(using Ab, ab, etc., to denote the number of AB observed, of ab observed, etc.), for, substituting the above values of AB, ab, etc., in this formula, we obtain

$$\sqrt{\frac{krA'B' \times kra'b'}{ksA'b' \times ksa'B'}} = \sqrt{\frac{k^2r^2A'B'a'b'}{k^2s^2A'b'a'B'}} = \sqrt{\frac{r^2}{s^2}} = \frac{r}{s}.$$

This formula should be used only when the smallest class has not a very large probable error, for, by multiplying the value of this class in the formula, we give the entire result a probable error proportional to that of the smallest class. Another objection to the formula is that it assumes that each factor produces the same specific lowering of viability, independently of whatever other factor it comes into combination with; this is not always true, since factors often produce different effects when in different combinations.

The two difficulties encountered above are largely avoided by the second method, which involves making two different kinds of crosses in preparation for the linkage determination: *i. e.,* cross AB by ab, and what may be termed the "contrary cross," Ab by aB. A back-cross of the F_1 from the first cross gives the gametic ratio r (AB) : r (ab) : s (Ab) : s (aB); and the other cross results in gametes showing the proportion s (AB) : s (ab) : r (Ab) : r (aB). Suppose that w per cent. of AB individuals are viable, x per cent. of ab, y per cent. of Ab, and z per cent. of aB. Then in the first cross the observed ratio would be r w (AB) : r x (ab) : s y (Ab) : s z (aB), and, in the second cross, s w (AB) : s x (ab) : r y (Ab) : r z (aB). The numbers actually observed in the crosses would be some multiple of these ratios, but a different multiple in the two cases. Thus we could designate the numbers actually observed in the first cross as krw(AB) : krx(ab) : ksy(Ab) : ksz(aB), and the numbers in the second cross as csw(AB) : csx(ab) : cry(Ab) : crz(aB).

In this case the ratio r : s may be obtained by the following formula:

$$\sqrt{\frac{AB_2 \times Ab_1}{AB_2 \times Ab_1}}$$

(using the symbol AB_1 to denote number of AB observed in the first cross, Ab_2 to denote number of Ab observed in the second cross, etc.). Now, the value of AB_1 has already been given as krw, of Ab_2 as cry, etc. Substituting these values in the above formula, we obtain

$$\sqrt{\frac{krw \times cry}{csw \times ksy}} = \sqrt{\frac{r^2kcwy}{s^2ckwy}} \doteq \sqrt{\frac{r^2}{s^2}} = \frac{r}{s}.$$

Besides this formula involving AB and Ab, there are three similar formulas which will also give the gametic ratio, namely:

$$\sqrt{\frac{AB_1 \times aB_2}{AB_2 \times aB_1}}; \qquad \sqrt{\frac{ab_1 \times Ab_2}{ab_2 \times Ab_1}}; \qquad \sqrt{\frac{ab_1 \times aB_2}{ab_2 \times aB_1}}.$$

That formula should usually be chosen which contains the largest number of individuals in its smallest class, for this would usually have the least probable error.

This method makes no assumption as to an independent action of the different factors in reducing viability. It does assume, however, that for individuals with the same combination of factors there is the same degree of viability in the two experiments; this is not always true, since under different conditions of food, etc., individuals of the same genetic type may have very different degrees of viability; moreover, there are sometimes "invisible" factors present in one experiment but not in the other which influence viability and which are linked with the factors that are being studied. The assumption, nevertheless, is unavoidable. But it can be shown mathematically that any errors in the calculated values, due to assumptions made in following the formulas of either the first or the second method, are greatly reduced by using a combination of the two methods; namely, by making "contrary crosses," calculating the linkage value in each of them by means of the first method, and then taking the square root of the product of these two values.

In a cross involving three or more factors no formula corresponding to the one first given is possible, and before it is possible to use a formula corresponding to the second method, an increasingly large number of different kinds of crosses must be made, according to the number of factors involved. Still another method is, therefore, necessary in order to obtain fairly accurate results from crosses involving many factors, except in the rare case that these factors have very little differential effect on viability. The method devised is as follows:

The female, heterozygous for many factors, whose gametic output it is desired to study, is back-crossed, not to a multiple recessive male, but to one homozygous for all, or nearly all, the *dominant* factors (these are, in the case of flies, mostly the normal allelomorphs). All the offspring appear alike, then, in that they all show the dominant characters of their father (except in the case of sex-linked factors, which are transmitted by the father to his daughters only), and so all should be of the same viability, except for the insignificant effect of the recessive factors

present in heterozygous condition (and the effect of the one or two characters wherein the father may not have been dominant). Thus error due to differential viability may be held within safe bounds.

It may be objected, however, that we have, as it were, killed the patient in curing the disease—that there is no use in overcoming the discrepancies in the count due to differential viability, if we thereby eliminate the possibility of making any count at all, by making all the offspring appear alike! It is true that, in such an experiment, it is impossible to tell *by inspection* of any offspring, what maternal factors were present in the ova from which they sprang, since these factors are made invisible, so to speak, by the dominant factors brought in by the sperm. But the factorial composition of each of these offspring (which we will for convenience call "F_2") can be determined by breeding tests. The plan which was followed was to mate the F_2 flies, each in a separate bottle, to individuals containing the recessive factors. Thus whatever recessive factors were present in the eggs of the original heterozygous female ("F_1"), whose output it was desired to test, would become visible the generation after (in "F_3"). Whereas, in an ordinary linkage determination, each bottle produces a large number of flies, which need merely be classified according to their appearance, and counted—in this case, each of the offspring themselves requires to be mated and given a whole bottle to itself, and its progeny in turn ("F_3") must be examined. In other words, in ordinary cases, there is only one bottle necessary for a count of many flies, but in this case one bottle represents one fly of the count. The numerical relations existing between the flies ("F_3") hatching in one of these final testing-out bottles need not be determined, however; that is, these flies need not be counted; all that is necessary is a "qualitative" determination of what recessive characters appear among them, in order to judge of the composition of their parent (F_2), which is the fly recorded in the count. Thus far 1008 of these test bottles have been recorded.

In preparation for this experiment the main task was to

secure stock that contained many mutant, linked factors at the same time. But, as was explained in the account of experiments with the third chromosome, it is necessary, in dealing with linked factors, to make the crosses in a particular way to secure a "multiple stock." Thus, it may be pointed out again here, a stock containing factors A, B, C cannot be obtained ordinarily by crossing stock A to stock C, and then crossing the double stock A C (produced in F_2, F_3, or F_4 from the first cross) to stock B; because it would require double crossing-over for the hybrid fly, containing A and C in one chromosome, and B in the other, to produce a gamete with A, B and C all in the same chromosome (assuming the factors to be linked in this order). If the linkage is tight such double crossing-over will never occur. But by first obtaining stock A B and then crossing this to C, stock A B C may be secured; for in the hybrid fly that contains A B in one chromosome and C in the other, a crossing-over between B and C will result in a chromosome that contains A, B and C, the link between A and B not having been broken.

In other words, the factors can only be added together in a certain order, owing to their position in the linkage chain. Just as in adding links to a chain, one or more factors cannot be wedged in *between* factors in another collection (except by double crossing-over); but if they lie *beyond* this collection, they may be added on, either singly or in a group. The information that had already been gained by Sturtevant, Morgan and Bridges concerning the order in which various factors lay, was therefore of great service in determining how the crosses should be made, to get the factors together, and besides this several double stocks of a sort that could be used in the present experiment had already been synthesized by them. But the progress of the experiment was very considerably retarded by the fact that the position of a number of the factors which it was desired to use had not yet been determined. These comprised bifid and forked in chromosome I and dachs, jaunty, curved, arc and balloon in II. (The exact position of jaunty with respect to black, and of

balloon with respect to speck is still unknown.) Various "trial and error" matings were therefore made in the hope of getting these unplaced factors in suitable combinations, and crosses were also undertaken to secure such data in regard to their position as would be useful for the purpose in view. These attempts were often cut short, owing to the information which was meanwhile being accumulated by the other workers, but before the latter information was obtained the positions of bifid, forked and dachs had been determined, and several multiple stocks that were later used had been made up.

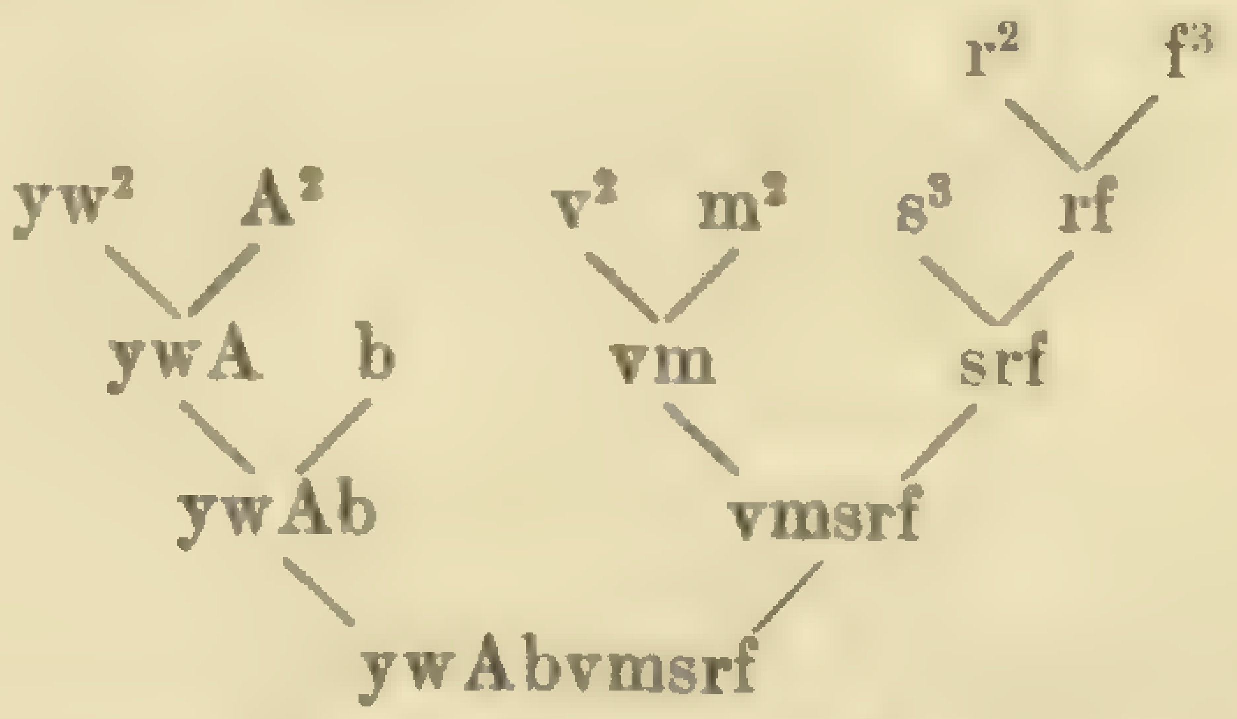
We may now consider specifically what combinations of factors were actually employed in the experiment and what special methods were used for securing and maintaining these combinations.

In the case of the first chromosome, it was desired, for the final linkage determination, that the heterozygous flies, whose gametic output was to be tested, should contain most of the recessive factors (which are usually the mutant ones) in one chromosome, and the dominant (usually normal) factors in the other, for it was considered worth while to test the possibility that a chromosome containing so many mutant factors might behave abnormally. Furthermore, if the commonly accepted belief were correct, that recessive factors are "absences," it was possible that the chromosome with many recessive factors might be shorter than the other, and that linkage disturbances might arise for this reason. Two stocks were, therefore, made up, of the factors in the first chromosome, to supply these two kinds of chromosomes for the heterozygous females to be tested. One stock contained the mutant factors for yellow body color, white eyes, abnormal abdomen, bifid wings, vermilion eyes, miniature wings, sable body color, rudimentary wings, and forked spines. All of these factors are recessive except abnormal abdomen, which is only partially and irregularly dominant. The other stock contained only the mutant factors cherry eye, club wing, and bar eye. Cherry is an allelomorph to the factor for white eyes carried by the other chromosome,

and is dominant to it, though not completely; club is recessive; bar is dominant (somewhat incompletely).

The reason that club was not put into the series with the other recessives is that it was discovered (by Bridges) after this series had already been put together, and so it would have required taking the stock apart again, or else obtaining a rare double cross-over, to wedge club into this series. It was a valuable factor to have in the experiment, however, since it lay in a region of the chromosome where there were no other mutant factors to give data as to crossing-over. Accordingly, it was inserted in the other series. It will be observed, however, that, in spite of this, one chromosome contains 7 more dominants than the other.

The order of the above factors is y, w or c, A, b, c_1, v, m, s, r, f, B_r. In making up the first stock the factors were put together as follows (omitting from consideration all trial or discarded combinations):



Of course the putting together of factors from two stocks, although shown above as only one step in each case, always requires several generations. Moreover, as will be seen below, these steps do not usually consist in getting the ordinary F_2 or back-cross, in the case of the complicated combinations. This is partly because of the serious obstacle which the poor viability of flies having many mutant characters presents to the making up of multiple stock, just as it does to the securing of counts from it; moreover, in the making up of stock, the sterility of such flies is an equally important difficulty.

These difficulties were overcome here in much the same way as they were in making the counts—namely, by keeping the stock, so far as possible, heterozygous. For ex-

[2] From Morgan.

[3] From Bridges.

ample, in the last step shown in the preceding diagram, where factors ywAb and vmsrf are to be put together, it was found that females of both of these kinds were extremely difficult to keep alive. It was, therefore, decided to mate a vmsrf male by a female which contained ywAb in one chromosome and normal factors in the other. Such a female would be easy to breed from, as the normal factors dominate. About half the daughters (let us call them F_1) would be of composition $\frac{\text{ywAb}}{\text{vmsrf}}$ (representing the mutant factors in the maternally derived chromosome on the upper line, those from the father on the lower line). All the daughters (F_1) would, however, appear normal, but if these F_1 females were bred in separate bottles, those of the desired composition $\frac{\text{ywAb}}{\text{vmsrf}}$ would be distinguishable from the others by their offspring (F_2). All bottles in which the parents (F_1) had not been of the desired composition could then be discarded. Next, among the offspring (F_2) of those females which proved to be of composition $\frac{\text{ywAb}}{\text{vmsrf}}$, it was necessary to select the ones which, by reason of crossing-over between b and v, contained all nine factors in the same chromosome (*i. e.*, ywAbvmsrf). But such individuals, if homozygous, never live long enough to mate, so great is the lowering of viability produced by all these mutant factors at once. Consequently, some method must be used of obtaining in this cross heterozygous individuals (F_2) which received this cross-over "nontuple" chromosome from their mother, and of distinguishing these from other individuals produced by the cross. The natural suggestion would then be that the F_1 females should be mated by normal males, and the F_2 which receive this cross-over chromosome could then be distinguished by breeding tests as their mother had been. The crossing-over desired, however, does not occur in more than one eighth of the flies, and so breeding tests designed to be certain of securing at least one individual of the required composition would have to be rather extensive. In this case, however, the desired F_2

flies can be "spotted" in another way, without breeding tests, and yet without making them homozygous for many mutant factors and thus inviable. The method used was to mate the F_1 females to bv males, which had been made up for this special purpose. The b v daughters (F_2) must be cross-overs, since in the F_1 mother b and v were in different chromosomes; moreover, a glance at the formula of the F_1 females will show that these cross-over chromosomes must have been formed of the left-hand end of the upper chromosome and the right-hand end of the lower. Thus these bv females contain a chromosome with all nine mutant factors (except in the case of the few double cross-overs). Since, however, they were homozygous in only two mutant factors, they could easily be bred.

A similar scheme was used in many of the other steps shown in the diagram representing combinations made in group I, and was also used frequently in group II. Owing to the fact that rudimentary winged females (group I) are practically sterile, devices of this sort had to be used in dealing with flies containing this factor from the very start, and the same may be said of flies with dachs legs (group II), since these also were found very hard to handle. In most of the other cases, however, it was not necessary to use such a method before several factors had been combined together, as flies homozygous for just two or three mutant factors were generally viable enough to handle. There would be no object in wearying the reader with a description of the exact way in which each of the steps was taken; it is the author's purpose only to explain the nature of methods used, giving only sufficient examples to make clear the details of any devices never previously employed that might be capable of application to other cases.

From the example of the cross involving bv, previously given, we may now generalize, and establish the rule that in making up, and also in keeping stocks containing many linked recessive factors, if the latter cause a marked lessening of fertility or viability, it is best to follow the practise of keeping the stocks heterozygous, by back-crossing them to stocks containing only the few recessive (or par-

tially recessive) factors necessary to show which offspring contain the desired cross-over or non-cross-over chromosome. In the example, this method was used in combining two stocks to make up a recombination stock. The same means is employed in maintaining the multiple stock after it has been synthesized. Thus, in the case of group I, the females containing in one chromosome the combination ywAbvmsrf (the "F_2" obtained above), were crossed to cc_1B_r males. In this way some daughters (F_3) are produced (which these were was determined by breeding tests) that received from their mother ywAbvmsrf, and from their father cc_1B_r. These F_3 females having the composition $\frac{\text{ywAbvmsrf}}{cc_1 \quad B_r}$, were then back-crossed to cc_1B_r males again, in order to maintain the stock. Since all the daughters (F_4) received cc_1B_r from their father, those which do not show these characters fully developed must have received from their mother factors near both ends of the chromosome containing the nine mutant factors. Therefore, except for the very few flies in which crossing-over occurred between w and y, which is at the very end, or in which double crossing-over occurred, all the light cherry, normal winged, partially bar eyed flies will have a composition like that of their mother, and may be bred in the same way, again to the cc_1B_r males, which now hatch from the same bottle. This then is a cross exactly like the preceding one, except for the few cross-over flies above mentioned. The latter may be detected, however, and their offspring discarded if the females are bred in separate bottles. This same cycle may be repeated generation after generation. Thus a continual supply is maintained of flies heterozygous for all these factors.

In making the linkage determinations, such flies are bred to normal or to bar males, and the female offspring, which are all alike in appearance except in respect to the partially dominant factors A and B_r, and which should, therefore, have had approximately equal chances for surviving, are individually tested for their contained characters. For the tests, the female need not be virgin, since, whatever kind of male is employed, the sons will show only

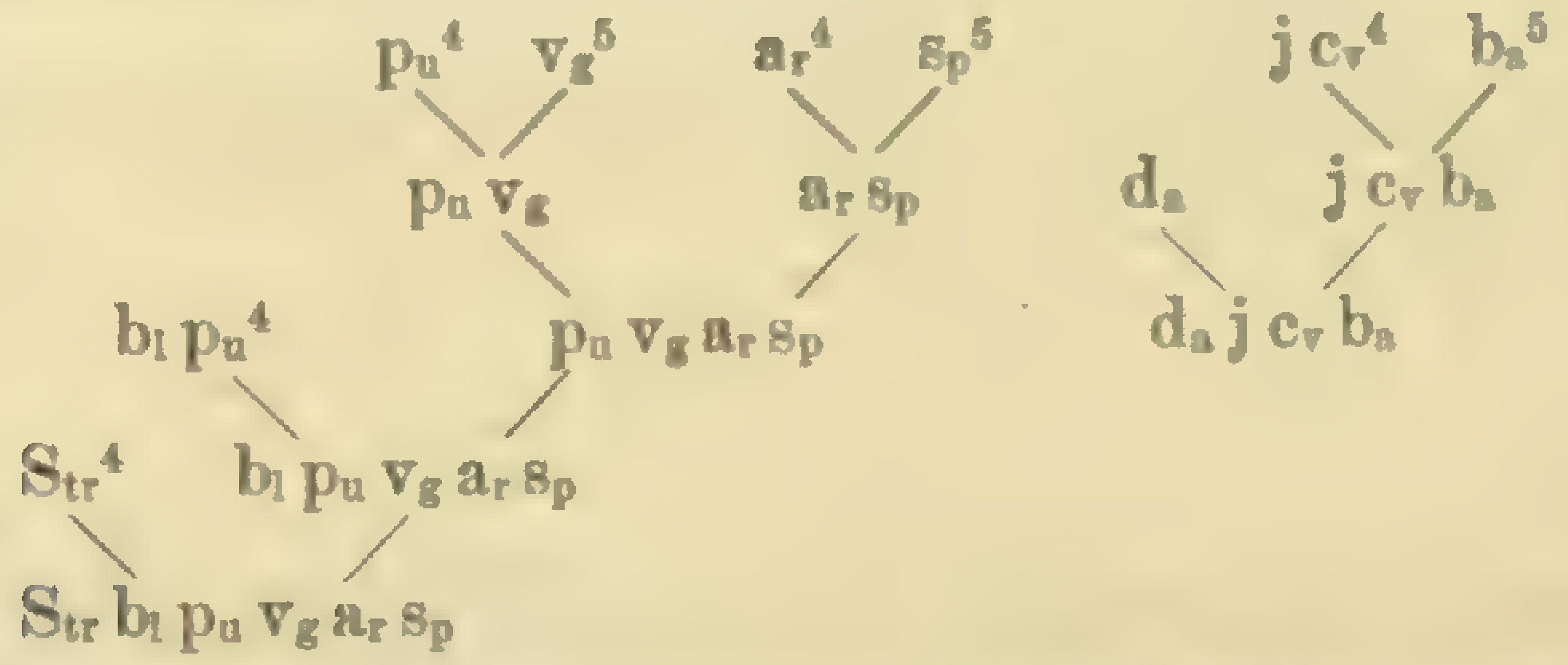
those sex-linked characters that their mother contained and they may therefore be used to determine the composition of their mother. As a matter of fact, however, males containing v were generally employed, so that v, if it had been present in the tested female, would appear in her daughters as well as her sons. This additional test for v was desirable because it is a factor which in a white, cherry, or bar eye it is difficult or impossible to detect.

Stock of the second chromosome was obtained, and is maintained, in an essentially similar way. Here the attempt was not made to put most of the mutant factors into one of the two chromosomes of the heterozygous females to be tested. This was partly because an experiment of this sort with one chromosome would seem sufficient. Moreover, it was harder to make up multiple stocks of the second chromosome, since the order of certain factors had not at first been well determined, and since, besides, it takes a greater number of generations to put non-sex-linked factors together into the same stock than it does to put sex-linked factors together. For, if two recessive stocks of chromosomes II are crossed, the F_1 males, in which crossing-over never occurs, transmit the recessive factors of only one stock to each son and daughter. The latter then can not be homozygous for both sets of recessive factors, and so it is impossible to pick out, except by further breeding, those that received both sets from the mother. But as in the case of the bv illustration given, if a male with C D is available to cross with the "F_1" hybrid female $\frac{ABC}{\quad DEF}$, the "F_2" individuals showing both characters C and D must have the composition $\frac{ABCDEF}{CD}$, and so the desired cross-overs may be picked out immediately in F_2.

A somewhat similar scheme, often especially useful for obtaining desired combinations in a non-sex-linked group, involved making use of cross-overs that break combinations already obtained. This too may be shown by an example. It was desired to obtain a stock containing the second chromosome factors dachs legs, jaunty wings, curved wings, and balloon wings. Dachs black stock al-

ready had been made up, as had $j\,c_v\,b_a$. These two stocks were crossed together, and the F_1 was back-crossed to $d_a\,b_l$. In the F_2 generation all the dachs that are *not* black are cross-overs in the region between these two factors, and so must contain, in the same chromosome with dachs, instead of black, the factors $j\,c_v$ and b_a, for black is in such a position in the chromosome that a cross-over between d_a and b_l must nearly always be a cross-over between d_a and $j\,c_v\,b_a$.

In the case of the second chromosome the individuals tested for linkage contained, in one chromosome, the factors streaked thorax, black body color, purple eyes, vestigial wings, arc wings, and specked thorax, and in the other chromosome, the factors dachs, jaunty, curved and balloon. The order of these factors is as follows: $S_{tr}\,d_a\,b_l\,p_u\,v_g\,c_v\,a_r\,s_p\,b_a$. The way in which they were combined is as follows:



As $d_a\,j\,c_v\,b_a$ is very inviable it is kept in heterozygous condition by back-crossing, in each generation, normal appearing males of the composition $\frac{d_a\,j \quad c_v \quad b_a}{b_l\;p_u\,v_g\,a_r\,s_p}$ (called $\frac{4}{5}$ for short) to $b_l\,p_u\,v_g\,a_r\,s_p$ females (called 5). Since there is no crossing-over in the male, all the offspring are either apparently normal, $\frac{4}{5}$, or the homozygous quintuple recessive, "5." The same process can then be repeated in every generation, by crossing the normal-appearing sons to their recessive sisters. It is evident that the "5" females which are used need not be virgin, as they could have been fertilized only by $\frac{4}{5}$ or by 5 males. When $\frac{4}{5}$ males are crossed by 5 females which have been made up so as to contain in addition the dominant factor streak (these 5 females need only be heterozygous for streak; homozygous streaks are hard to handle), the daughters

[4] From Bridges.

[5] From Morgan.

which appear streaked but otherwise normal, must have the composition: $\frac{d_a\ j \quad c_v\ \ b_a}{S_{tr}\ b_1\ p_u\ v_g\ a_r\ s_p}$. These are the "$F_1$" females whose gametic output is to be tested. Accordingly, they are crossed to normal males. All the offspring ("F_2") appear normal (except for the dominant, streak), but the factors they received from their mother may be determined by mating them, individually, to ⅘ males, for the latter contain (heterozygous) all recessive characters possible in the former.

It was at first thought that labor might be saved, and certain points in addition determined, by conducting the linkage determinations on flies heterozygous for the factors used in both chromosomes I and II at the same time, instead of making determinations of the linkage in the two chromosomes in separate experiments. The multiple stocks of the two chromosomes were, therefore, crossed together, and females were finally obtained that had the composition:

$$\frac{y\,w\,A\,b\,v\,m\,s\,r\,f \qquad}{cc_1 \qquad B_r} \quad \frac{S_{tr}\ b_1\ p_u\ v_g\ a_r\ s_p}{d_a\ j \qquad c_v\ \ b_a}.$$

These females, heterozygous for 22 mutant factors, were then crossed to normal males, and the composition of their female offspring was tested by mating these in separate bottles to ⅘ males. The maintenance of the double-multiple stocks proved to be extremely difficult, however, and so, after obtaining determinations for 166 offspring from such females, the two groups of mutant factors were again separated. The data obtained in this part of the experiment show that there is no linkage of any of the twelve factors studied in group I with any of the ten studied in group II; this is of course in marked contrast to the relations shown between factors in the same group. The conclusions of previous workers that no factor in one group was linked with any factor in another group were based on results obtained with comparatively few combinations of factors, which were chosen as samples, so to speak. It will be seen that in the present work these conclusions have been confirmed by a study of 132 differ-

ent combinations of factors in group I with factors in group II.

Classification of Factor Combinations transmitted by females having the Composition: $\frac{\text{y w A b v m s r f}}{c \quad c_l \quad B_r}$

	Yellows		Grays		Totals
	Non-cross-overs				
	ywAbvmsrf	186	*c* c_l B_r	200	386
Between	*Single Cross-overs*				
y and w	*yc* c_l B_r	2	*wAbvmsrf*	5	7
w and A	*yw* c_l B_r	3	*cAbvmsrf*	5	8
A and b	*ywA* c_l B_r	4	*c bvmsrf*	11	15
b and c_l	*ywAb* c_l B_r	17	*c vmsrf*	27	44
c_l and v	*ywAb* B_r	46	*c* c_l *vmsrf*	51	97
v and m	*ywAbv* B_r	7	*c* c_l *msrf*	9	16
m and s	*ywAbvm* B_r	18	*c* c_l *srf*	19	37
s and r	*ywAbvms* B_r	28	*c* c_l *rf*	38	66
r and f	*ywAbvmsr* B_r	0	*c* c_l *f*	5	5
f and B_r	*ywAbvmsrf* B_r	0	*c* c_l	1	1
Between	*Double Cross-overs*				
y and w; c_l and v	*yc* c_l*vmsrf*	1	....		1
y and w; m and s	....		*wAbvm* B_r	1	1
y and w; s and r	*yc* c_l *rf*	1	*wAbvms* B_r	1	2
y and w; r and f	*yc* c_l *f*	1	....		1
w and A; c_l and v	*yw* c_l*vmsrf*	1	....		1
w and A; r and f	*yw* c_l *f*	1	....		1
A and b; c_l and v	....		*c b* B_r	1	1
A and b; s and r	*ywA* c_l *rf*	1	....		1
b and c_l; m and s	*ywAb*c_l *srf*	1	*c vm* B_r	1	2
b and c_l; s and r	*ywAb*c_l *rf*	4	*c vms* B_r	3	7
c_l and v; v and m	....		*c* c_l *v* B_r	1	1
c_l and v; s and r	*ywAb rf*	7	*c* c_l *vms* B_r	1	8
c_l and v; r and f	*ywAb f*	2	....		2
c_l and v; f and B	*ywAb*	1	....		1

Total Double and Single Crossing-over

Between	Observed Number	Per Cent. of Crossing-over	Between	Observed Number	Per Cent. of Crossing-over
y and w	12	2	v and m	17	2
w and A	10	1.5	m and s	40	6
A and b	17	2	s and r	84	11.5
b and c_l	52	7.5	r and f	9	1.2
c_l and v	112	16.	f and B_r	2	0.3

Not only the independence of the factors in the two groups was shown by this experiment in which the two groups were followed at once, but also the independence of the crossings-over. In the total of 166 cases, there were 81 in which chromosome I underwent crossing-over (either

single or double), and 101 in which chromosome II crossed over. If it was a matter of pure chance whether or not crossings-over occurred in I and II at the same time, coincident crossing-over should have happened in $\frac{81}{166} \times 101 = 49+$ cases. The actual number of cases in which crossing-over occurred in both chromosomes at once was 52. Thus there is neither interference nor synchronism of these crossings-over, and this result too is strikingly dissimilar to the relations found between two crossings-over in the same chromosome.

Since the results in the two chromosomes were found to be independent in all respects, it is deemed unnecessary to list here all the different combinations which were found of factors in group I with factors in group II, and their observed frequencies. The results for the two chromosomes may more advantageously be separated and added to the other results, obtained when groups I and II were followed in different crosses.

The data for the first chromosome are given in the tables which follow. In all, 712 offspring of females heterozygous for the 12 mutant factors in group I have been tested.

The above results give a direct demonstration of the fact that the factors behave as though they are joined in a chain; when interchange takes place, the factors stick together in sections according to their place in line and are not interchanged singly. The fact is shown so patently as to require no further comment.

Non-crossing-over occurs in this chromosome in 54.4 per cent. of cases, single crossing-over in 41.7 per cent., double crossing-over in 4.2 per cent. No triple crossing-over was obtained in this count, although one, which will be described later, was obtained in the next generation, in one of the "testing out" bottles.

(To be concluded)

SHORTER ARTICLES AND DISCUSSION

THE CAUSE OF THE BELIEF IN USE INHERITANCE

This note expresses an effort to view the old and recurring problem of use inheritance from the aspect of the underlying motives of thought involved instead of through a consideration of the evidence directly bearing upon it.

The heredity of acquired traits is, theoretically, biological heresy. But the interminable cropping out of the belief even in professional circles indicates a strong psychological impulse toward the conviction. The mainspring of this impulse thus becomes a matter of some importance to the student of heredity.

To begin with, it is well known that the lay public almost without exception takes use inheritance for granted. Even evolution, in the real mental workings of most educated but unprofessional people, is more generally explained, unconsciously and in concrete cases, by appeal to the machinery of use inheritance than to that of selection. The phrases struggle for existence and survival of the fittest have indeed evoked a wide popular response on account of their picturesqueness, but their concepts are still but little employed, even in the intelligent and studied folk mind, as a real means of understanding or explaining evolution.

Those sporadic but in the aggregate numerous biologists who adhere to the doctrine of use inheritance, revert to it, or evince symptoms of a leaning toward it, may be divided into two types. The first class, probably because they think more penetratingly than the average, long ago perceived the inadequacy of selection alone as an explanation of organic evolution; and more lately perceive also the insufficiency of selection with mutations and Mendelian phenomena superadded. To students of this type, use inheritance is therefore merely a last resort, a hypothesis on which they fall back in default of any other to stop a logical gap. The only methodological criticism that can be made of this school is that it would undoubtedly be more stimulating of new discovery if we were frankly to avow the limits of our knowledge and leave certain things unexplained, than to complete the mental structure of evolution by piecing in a principle which admittedly rests only on contested facts and has opposed to it about as large a body of evidence as can be assembled on behalf of any negative and therefore logically unprovable proposition.

The second class consists of biologists and utilizers of biological material whose keenness of thought is below the average. This school introduces use inheritance into the conception of evolution because it has failed to comprehend adequately the essential problems of evolution, and approaches them substantially in the attitude of the layman.

The latter class is therefore merely unscientific and popular in its thought processes; the former, having exhausted scientific means and found them inadequate, returns, more or less frankly in despair, to current folk opinion. The problem accordingly is to discover the basis of the deeply rooted popular notion that is involved in both cases.

While never formulated into a definite working principle until Lamarck, because of the world's lack of specific scientific interest in organic phenomena, the principle of use inheritance has nevertheless been tacitly assumed by civilized nations of all periods, and is taken as self-evident even by savages. It must therefore rest on a large mass of common experience interpreted by an elementary process of thought. Such an elementary process—in fact the only elementary process of wide scope—is analogy.

The question then becomes what may be the basis—real enough though unscientifically employed—for the analogizing that has resulted in the conviction that use heredity exists. There must evidently be a broad group of phenomena in human experience that bear some resemblance to the hereditary transmission of the acquired.

These phenomena are the exceedingly common ones of social inheritance or cultural transmission and growth. We do "inherit" a name, or property, or knowledge of a language, or the practice of an art, or belief in a particular form of religion. Biologically such "inheritance" is of course absolutely distinct from "heredity" because the mechanism of transmission is different. The source of social inheritance is not restricted to parents and actual ancestors in the line of descent, but embraces a multitude of individuals, consanguineous and unrelated, dead, living, and sometimes even junior to the inheritors; in other words, the totality of the social environment, past and present, of an individual. We can and do "inherit" property from an uncle, our "mother tongue" from a nurse, the arithmetic evolved by past ages from a schoolmaster, our dogmas and philosophy from a prophet, our political and moral beliefs from the whole circumambient public opinion.

As this social or cultural transmission concerns human beings, it is of more immediate interest to the normal unschooled mind than the transmission which gives organs, instincts and peculiarities to animals and plants. It is therefore recognized much sooner than the processes which guide biological or organic transmission. It needs no proof that in his development man was concerned far earlier with himself than with animals or other parts of nature. It is well known, for instance, that the animism which is accepted as the basis of all religion, anthropomorphizes not only its gods and the vaguer forces of nature, but especially animals, plants and objects.

It is only recently, accordingly, that the world has paid any true attention to organic heredity, whereas since the beginning of human existence there has been recognition of social inheritance. History, the science of human society, is, even in a relatively advanced form, several thousand years old, and as a rudiment has enough interest to appeal to savages. Biology, the science of the organic, has an age of barely two centuries.

It is significant that the first theory of organic evolution, that of Lamarck, resorted wholly to the explanation of use inheritance borrowed from social inheritance. A second stage was reached when Darwin introduced the organic factor of selection, though refusing to break with the older explanation. A last phase was inaugurated when Weismann insisted that organic phenomena must be interpreted solely by organic processes.

The priority of reasoning by analogy over reasoning by means of a specific mechanism is a world-wide historical phenomenon. The two modern views of evolution and creation are found as crude cosmic philosophies in the mythologies of the most primitive savages, as well as in the thinking of Hindus, Semites, Greeks, and Romans. But they occur, one as an analogy with the familiar phenomenon of manufacture or making of objects by hand, the other as an analogy with the equally familiar phenomena of birth and growth. What modern science has done is to adopt these age-old and crude ideas, as it has adopted the half-mythologic concepts of the atom and ether, and put them to new use. Only the uneducated think of Darwin as the originator of the doctrine of evolution. What he originated was an organic and in his day new mechanism, by which the old concept of evolution could be explained and therefore supported.

The distinction between the social and the organic is far from

a novel one. But the two groups of phenomena, and the processes involved in each, are still very frequently confounded in other domains than that of use inheritance. The whole eugenics movement, for instance, so far as it is a constructive program and not a mere matter of ordinary practical prophylactic social hygiene, rests upon the assumption that social progress can best be accomplished by organic means. It may be rash to deny wholly that such an end can be achieved in this way or that it would be useful. But the orthodox eugenist, from the time of the founder Galton, has consistently and complacently made this assumption without any inquiry as to its justification. Lamarck erected a false doctrine of evolution through explaining the organic in terms of the social, or in terms derived by mere analogy from the social. The eugenists of to-day, it may fairly be suggested, bid fair to vitiate a movement that springs from the most sincere of motives, by resting its basis on an interpretation of the social as merely organic.

In summary, the doctrine of the hereditary transmission of acquired characters is no more disprovable than it is provable by accumulation and analysis of evidence. It springs from a naïve, unscientific, and even primitive method of reasoning by analogy, which in this case works to a confusion of the long-distinguished and necessarily distinct concepts of the organic and the social. The doctrine must therefore be dismissed on purely methodological grounds. It is possible that when the missing factor or element of evolution is discovered that neither Darwin nor the mutationists have been able to find, this factor will prove to be something superficially similar to use inheritance. But it will differ from the present only half-discredited but discreditable factor of heredity by acquirement, in containing an organic mechanism, and will therefore be essentially different from this crude and confused assumption.

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TRIFOLIUM PRATENSE QUINQUEFOLIUM

Hugo De Vries in his mutation theory tells us in detail about his production, by means of selection from two mutant forms, of a five-leaved race of red clover. This race he called *Trifolium pratense quinquefolium*. The two plants obtained for starting

his selection were found, according to the author, growing near the edge of a road that was covered with grass. He does not tell us the exact composition of all the leaves of these two plants with which he started, but states that they bore several tetramerous and one pentamerous leaf. Neither of the plants, therefore, could be called mutants of a new race, but were mutating forms from which De Vries obtained, after a process of most rigid selection, his highly variable race, *Trifolium pratense quinquefolium.*

During the spring of 1914, I found growing in an old orchard at Corvallis, Oregon, a red clover plant that showed "full-fledged" all the characters of *Trifolium pratense quinquefolium* about which De Vries has written, and which took him so long to obtain by the aid of selection. This clover plant, after careful examination, was transplanted in one of my experimental plots for further study. The following descriptive notes of it are given: Of medium height; good color; normal as to vigor, but not luxuriant; seven stalks; leaves, 4 trimerous, 5 tetramerous, 12 pentamerous; total number of leaves, 21. Not only did the pentamerous condition of so many leaves represent the mode for leaf variation, but there were more five-leaved leaves than both four-leaved and three-leaved leaves combined.

The magnitude of this mutation may be more fully appreciated when we reflect that De Vries, after selecting for three generations and obtaining 300 plants, found only one that gave as high a percentage as 36 for both tetra- and pentamerous leaves; while the percentage of tetra- and pentamerous leaves for all those counted, 8,366, was only 14.

After finding this specimen of *Trifolium pratense quinquefolium* I was exceedingly desirous of obtaining another plant with which to cross fertilize it so as to obtain a race which could be used commercially, but repeated searches made for many days failed to reveal any other plant suitable to cross with this one. Thus failing to find a second plant, I decided to propagate the discovered mutant vegetatively. This method gave some degree of success, and a few plants were reared during the summer of 1914 from slips. When I left Oregon at the end of the summer, four of these plants were transferred to a private lot, and a railing, supported by stakes, was put around them.

Examination on June 3 the following summer (1915) showed two of these slips doing well, one had been trampled on and

killed by a cow, and the other was dead. At this time I still had hopes of obtaining a race of five-leaved red clover, but when I returned to Oregon and examined these plants on June 20 all such hopes vanished, for a neighbor's cow had completely ruined them, cropping off all the stalks down to the ground. None of the plants revived after this last injury.

Records for the leaf production of this mutant were kept, and from them I have obtained the following: On May 11, 1914, a count was made of all the leaves produced up to date. There had been produced 6 trimerous, 7 tetramerous and 17 pentamerous leaves; 30 in all, over 56 per cent. pentamerous. We notice, however, a slight decline in the percentage of pentamerous leaves produced, since the plant was found. This decline, early noticed, continued throughout the summer, and on August 23, when I made my last leaf counts, I obtained the following record of leaf production for this mutant clover plant:

	Trimerous Leaves	Tetramerous Leaves	Pentamerous Leaves
Leaves on plant when found	4	5	12
Leaves produced since plant was found	30	11	17
Total leaves produced by plant	34	16	29

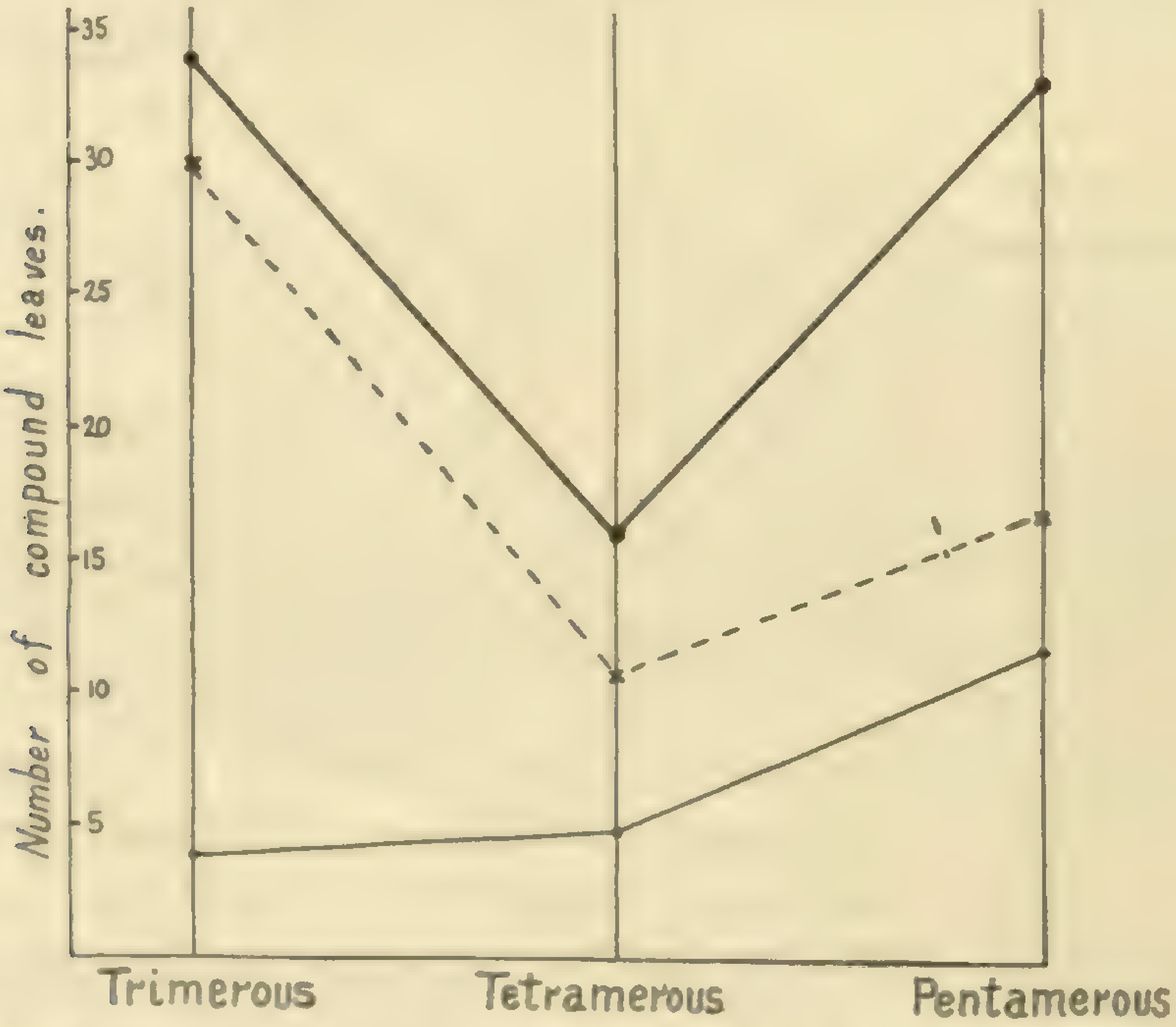

FIG. 1. Frequency polygon showing leaf variations of a mutant individual of *Trifolium pratense quinquefolium*. Light solid line shows variations of leaves on plant when discovered; dotted line, variations in leaves produced by plant in captivity; heavy solid line, variations for all leaves produced by mutant plant and its slip plant descendants.

How are we to interpret these results? Why should there be such a preponderance of pentamerous leaves produced during the early growth period of the plant, then a preponderance of trimerous leaves during the latter part of the season?

The records obtained for 1915 for the slip plants added but little to the 1914 records. The leaf production of slip plant No. 3, however, is interesting. In 1914 this slip plant produced 4 trimerous, 2 tetramerous and 4 pentamerous leaves. In 1915, however, it produced 0 trimerous, 0 tetramerous and 4 pentamerous leaves—showing strongly the inherited tendency to produce the pentamerous leaves during the second season. Clover heads were produced during the summer of 1914, but no seed was found in them.

No leaves were produced by this plant having more than five leaflets, a condition that obtained during the first three generations of De Vries's race, yet later he obtained both 6- and 7-merous leaves in abundance. A frequency polygon is plotted (see figure) for the leaf variations of this red clover mutant.

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NOTES AND LITERATURE

FAUNAL DISPERSAL[1]

THE widely different conclusions drawn by different students from the same facts is well illustrated by Matthew's recent paper on the geographic distribution of animals.[2] Although these differences are of many and varied sorts a fundamental one seems to be concerned with the question as to whether the peripheral part or the central part of the range of a group contains the more progressive members of that group.

The idea which is held, either consciously or unconsciously, by many is that after a group has arisen it spreads; and the migrants, meeting new conditions, develop new characters. These new forms then spread still further and develop other characters. Thus the more primitive members remain at or near the point of origin of the group; the successively more progressive members will be found at respectively greater distances from this point. According to this theory we may trace *backward* the dispersal of the group by following the distributions of the several members from the most progressive to the most primitive. We may not be able to follow this line to the actual point of origin of the group for the most primitive members may not be alive now and we may be unable to find their fossil remains. However, as organisms usually extend their range in more than one direction, we may be able to trace several lines of dispersal and deduce that the point of origin was near the place at which these lines tend to converge.

The other theory is as nearly the opposite as can be. According to it the first progressive makes its appearance well within the range of the primitive members of the group. If the new form is not an improvement over the primitive one, it dies out. If it be an improvement, it crowds the primitive form which is forced to leave its place of origin and migrate. It is sometimes made a part of this theory (but it is not a necessary part) that the reason a new form appeared among the old was that the new climatic conditions appeared there and that if the old form could, in its migration, keep in climatic conditions suited to it, well and good; if it could not, it either died out or became adapted to the

[1] Read at the February 15, 1916, meeting of the N. Y. Entomological Society.

[2] W. D. Matthew, 1915, "Climate and Evolution," *Annals*, N. Y. Acad. of Sciences, XXIV, pp. 171–318.

climatic conditions into which it was forced without, however, losing all the earmarks of its primitiveness. Later still, newer forms appeared at the old stand and forced their predecessors still further away. Therefore, if we follow the distribution of the several members of the group from the most progressive to the most primitive, we will trace the dispersal of the group *forward,* not backward.

It would probably be impossible to decide, either by logomachic methods or by watching present-day movements of species, which of these two theories is correct. However, we are not left without hope because paleontologists are daily digging up evidence which, when sufficiently complete and properly translated, will leave us in no doubt as to the history of the dispersal of certain groups, and there is little doubt that the same general principles which hold for those groups will apply to others concerning which we have not and probably never will have fossil record. Of course details may differ from group to group, but it is not probable that there is one set of laws for mammals and another for reptiles, one for birds and another for insects, that nature constructed a trans-oceanic bridge for one group, but stationed a guardian angel on it to prevent the passage of others. The chief differences probably were such as the differing responses of the different groups to changes of the environment and the differing powers of different groups in overcoming given barriers to dispersal.

Next to that brought about by the holding of the diametrically opposing theories just discussed, perhaps the most important source of confusion is in not keeping clearly distinct "center of origin," "center of dispersal" and "center of greatest development." They may all be in the same region or they may be as far removed from each other as the earth's surface will permit but they are not the same. A group may arise at *A* and move to *B,* from which point there are easy paths of migration to *C, D, E* and *F*. It may, and doubtless would, go to all of these but it might find that *E* alone furnishes good conditions for its future development. Its center of origin would then be at *A,* its center of dispersal would be at *B,* and its center of greatest development at *E*. According to the first theory discussed above, the more primitive forms would be found at *A* or *B* and the later developments at *C, D, E* and *F,* but chiefly at *E*. According to the second theory, *C, D, E* and *F* would have the primitive forms (although they might be much modified, especially at *E*) and the progressive members of the group would be at *A* or *B*.

Adams[3] did good service in bringing together a number of suggested "criteria for the determination of centers of dispersal." It was not to be expected, and doubtless Adams did not expect, that all of them would stand up under a test. Without attempting to exhaust the subject, present anything new or review all that has been said about them, certain notes may be made in connection with this discussion, taking up the criteria *seriatim.*

1. *Location of the Greatest Differentiation of the Type.*—I believe it is more than a mere question of definition to say that the offering of this criterion is an instance of the confusion of "center of dispersal" with "center of greatest development." Two of the stock illustrations of great differentiation are those of marsupials in Australia and lemurs in Madagascar. Since these groups are so greatly developed on the respective islands they should, according to this criterion, have spread out from these islands to the rest of the world. Unless the paleontological evidence, as brought together and interpreted by Matthew, is false, that was not the history of these cases. On the other hand, wherever their points of origin were, their ancestors got into the Holarctic region and then spread in various directions. Now, if this be true, their center of dispersal would be Holarctica, although their greatest differentiation *at the present time* is toward the other end of the world. Lest the quibble should be raised by others, it should be stated that the real fundamental center of dispersal of a group is its center of origin and that there are

[3] C. C. Adams, 1902, "Southeastern United States as a Center of Distribution of Flora and Fauna," *Biol. Bull.*, VII, p. 122. After the present paper had gone to the press I obtained through the kindness of Professor Adams, a portion of "An Ecological Survey of Isle Royale, Lake Superior" prepared under his direction and published (1909) as a part of the Report of [Michigan] Board of the Geological Survey for 1908. In this he takes up, again, these criteria. He says "It should be clearly emphasized that it is the convergence of evidence from many criteria which must be the final test in the determination of origins rather than the dependence upon any supposedly absolute criterion." He discusses the various criteria in greater detail than was done in his 1902 paper and adds, as another criterion, "Direction indicated by seasonal appearance; vernal suggesting boreal or montane origin and aestival as austral or lowland derivation." I regret that I did not have earlier access to this paper but as I did not base my present remarks on his 1902 contribution with the idea of criticizing it in particular but for the purpose of pointing out the danger of relying too confidently on such evidence, it is probably unnecessary to change the text of this article in order to meet the expanded discussion in the 1909 paper by Adams. This is especially true since I intentionally omitted, for the sake of brevity, reference to certain papers, such as the one by Tower (1906), which bear on the same subject.

other centers of dispersal wherever there are branches in the forward movement of the group; but I understand the usual meaning of "center of dispersal" to be the point or points from which the principal lines went out which resulted in the wide spread of the group.

2. *Location of Dominance or Great Abundance of Individuals.*—This criterion seems futile. A group may have moved in all directions from a given region and died out entirely in the region from which it moved. Nothing is more clearly established than the changeableness of geologic climate and hence of all environmental factors and we know of many instances where nothing is left of a group of organisms but fossil remains in the regions of their former abundance and a few living remnants in far-away, protected spots. If, as seems very clear, we can not believe that even the most populous of these havens marks the center of dispersal of such a group, neither can we apply this criterion with safety to any other group. The area of present dominance is merely that area, of all those now inhabited by a group, which is at the present time most suited to the group—unless, of course, it has arrived in a more suitable area so recently that it has not had time to develop its dominance.

3. *Location of Synthetic or Closely Related Forms.*—This criterion will be considered more in detail later, but it may be remarked in passing that the location of closely related forms is of little help in arriving at the center of dispersal unless we know whether these forms are more primitive or the reverse and unless, furthermore, we have selected the right one of the two opposing theories which were mentioned in the beginning of this discussion.

4. *Location of Maximum Size of Individuals.*—It is difficult to see why individuals should be larger at the center of dispersal than elsewhere. Possibly they may be larger the nearer they are to present-day, optimum environmental conditions, but this is probably not often, and certainly not necessarily, anywhere near the ancient center of dispersal.

5. *Location of Greatest Productiveness and its Relative Stability, in Crops.*—Adam says this criterion is very closely related to the second one. If so, it fails for the same reason. Also, it would seem that the latter part of this criterion conflicts with criterion number one—"greatest differentiation of type."

6. *Continuity and Convergence of Lines of Dispersal.*—This certainly ought to work, provided we follow the lines in the right direction. The difficulty is that north and south lines on a more

or less spherical world converge either way we go. Furthermore, if we place confidence in oceanic bridges which have been washed away, piers and all, the lines of dispersal are apt to be frequently discontinuous.

7. *Location of Least Dependence upon a Restricted Habitat.*—For example, certain plants and animals which, in the region of New York City, are found only in sphagnum bogs, such as those of the Jersey pine barrens, occur more widely distributed farther north. According to this criterion their center of dispersal would be in the north and this may be true. Again, lizards occur, in the region of New York City, only or chiefly in these same pine barrens, while farther south they run about wherever they can get sunshine. According to this criterion the ancient center of dispersal of lizards was somewhere near the equator, but this may not be true.

8. *Continuity and Directness of Individual Variations or Modifications Radiating from the Center of Origin along the Highways of Dispersal.*—This criterion is probably stated rather more fully than was meant. If we knew all this, the problem would be solved. Perhaps Adams meant that continuity and directness of modifications ("individual variations" gets us into the biometric-mutation discussion and that is another story) point out the highways of dispersal from the point of origin. His reference on the next page to Osborn's law of adaptive radiation indicates that this is the proper interpretation of this criterion and, if so, the present author has no quarrel with it except to point out once more that we are left in doubt as to which way to follow the lines.

9. *Direction indicated by Biogeographical Affinities.*—I am not certain as to what this means. I suppose a given group which is neotropical at the present time has biogeographical affinities with other present-day neotropical groups. If we know the centers of dispersal of the other groups we have a working hypothesis concerning the center of dispersal of the group in question. If this be what is meant, it seems to be probable.

10. *Direction indicated by the Annual Migration Routes, in Birds.*—This criterion is meant to apply only to birds and I fear we know too little concerning the intricate problems of bird migration to say whether their present-day routes of annual migration follow the route of ancestral dispersal or not. Probably they do not, as the birds would be expected to change their routes with changing environmental conditions. Furthermore, although it is believed that birds return to their ancestral home

to breed, this is not so firmly established as to leave no doubt about which is going and which is coming.

Adams clearly stated that these criteria are for use where "we do not have paleontological evidence in sufficient abundance to materially aid us," but I confess to a feeling that we must still depend on paleontology to give us the general laws of dispersal. It is for this reason that such papers as Matthew's seem to me so helpful. I regret that the organisms in which I am most interested did not leave more marks on the sands of time, but if the great majority of mammalian groups left records to show that they followed a certain set of lines of dispersal and the end result is of a given character, it seems worth while to compare the end results of the dispersal of other forms with those of the mammalian groups. If the comparison is close, the deduction that the lines of dispersal also are comparable seems not unsafe. It certainly seems unwise to construct trans-oceanic bridges where conservative geologists say there could have been none, especially if they must be made so tenuous that only insects, spiders, snails, earthworms, fresh-water fishes and such small fry can cross, mammals being forbidden.

Several of the criteria given by Adams which appear to fail in helping us discover the ancient centers of dispersal seem to be indicators of present or potential centers. Thus the first one: a region where there is a "great differentiation of type" within a group would seem to be a region prepared to send members of that group into all the world. If paths exist or chance intervenes, this group should be able to fit some, at least, of its many different types into the new environments which it encounters in its spread. If all the surrounding territory is already occupied by more successful competitors, that merely means that there are no paths for the dispersal of this group. One of the real, but sometimes overlooked, barriers to faunal spread is the presence of competitors. This, however, does not negate the idea that a region of "great differentiation of type" is a potential center of dispersal.

In somewhat the same way the region of "dominance or great abundance of individuals" is a potential center of dispersal. The case is not so clear; but it would seem that where there is a *relatively* large supply of individuals more could be spared for, or would be forced into, colonization efforts than where there are relatively few.

This suggests that what was the center of greatest development (especially in variety of types) became the center of dis-

persal, and that what is now the center of greatest development is or will be a new center of dispersal provided there are means of dispersal. There seems to be a genetic relationship between these three centers and, of course, at the beginning the center of origin is also the other two.

Little need be said here about means of dispersal except to point out what Matthew also emphasized, namely, that time is long and luck is real. Those of us who have been brought up on the doctrine of evolution by selection of "chance" variations should have a whole-hearted respect for that sometimes abused word. Those who believe there has been time enough for alert Nature to seize upon enough chances to differentiate 400,000 species of insects, for example, need not strain unduly in swallowing the notion that a very small proportion of these have been able to get across relatively short stretches of water without a bridge. Those who believe that Nature not only seized upon but made opportunities for the differentiation of species should have no trouble in discovering easier ways for her to help her creatures spread their range than by raising up long narrow portions of the ocean bed for a certain few to cross dry shod and then sinking it to the discomfiture of those which are not of the elect.

Insects probably get about as easily as any creatures because most insects fly or may be blown long distances and, furthermore, the majority have at least two distinct stages in their life cycle during which they remain inert and without the necessity of feeding. If mammals can reach islands not connected by bridges, surely insects of many kinds can. Matthew places great stress on natural rafts as a means of transporting mammalian fauna. After giving briefly a "series of facts and assumptions [which] may serve to give some idea of the degree of probability that attaches to the hypothesis of over-sea transportation to account for the population of oceanic islands" he says:

> If then we allow that ten such cases of natural rafts far out at sea have been reported, we may concede that 1,000 have probably occurred in three centuries and 30,000,000 during the Cenozoic. Of these rafts, only 3,000,000 will have had living mammals upon them, of these only 30,000 will have reached land, and in only 300 of these cases will the species have established a foothold.[4] This is quite sufficient to cover the dozen or two cases of mammalia on the larger oceanic islands.
>
> Few of these assumptions can be statistically verified. Yet I think that, on the whole, they do not overstate the probabilities in each case.

[4] Matthew apparently changed, between pages 206 and 207, his ideas concerning the probabilities. However, it is the general notion and not the actual figures which is important.

They are intended only as a rough index of the degree of probability that attaches to the method, and to show that the populating of the oceanic islands through over-sea transportation, especially upon natural rafts, is not an explanation to be set aside as too unlikely for consideration.

I confess to some haziness as to the probabilities here set forth, but, if they are anywhere near true, entomologists need not worry. In addition to their creatures not needing rafts as badly as do mammals, it is certainly probable that every, not "one in a hundred," natural raft big enough to be noticed and recorded by voyagers contained not one, but many, insects. Smaller rafts or even single trees might contain many individuals of several species and, since a single fertilized female gives birth to many offspring, the chance of a given species establishing itself on virgin soil is much greater than it is in the case of mammals. Furthermore, insects have been dispersing since before the Carboniferous. Many of the islands may not be that old, but this simply means that insects have had a chance at such an island since the first wavelets rippled about its uplifting peak. The one thing which may be comparatively disadvantageous to insects is that many of them are rather closely bound up in their food relations with certain plants, but this disadvantage is somewhat decreased by the fact that, if phytophagous insects are carried on natural rafts, their food plant is likely to be a part of the material which makes up the raft and both may be established together. The pros and cons are numerous and involved. It is a balancing of probabilities with the burden of proof on the side which claims the right to make over major features of the earth's surface in the face of contrary geological evidence.

If this be true for the scant fauna of oceanic islands, what shall we say of the suggested bridges, running this way and that, across the oceans for the purpose of connecting continental faunas and floras, especially in equatorial regions? Mercator gave us a map of the world so constructed that the longitudinal lines are parallel from the north pole to the south. Now the fact is that a degree of longitude equals approximately 111,300 meters at the equator, 104,600 meters at 20° latitude, 85,400 meters at 40°, 55,800 meters at 60°, 19,400 meters at 80°, and no meters at the poles. Therefore the equatorial distances on Mercator's projection are relatively far too short. On the globe or on a proportional projection in which a meter at the equator is as long as a meter in Alaska we see that north of the Tropic of Cancer in the eastern hemisphere lies a huge land mass consist-

ing of Europe, northern Africa and most of Asia. This mass almost touches at its northeastern corner the somewhat smaller mass of North America. The intervening space, Bering Strait, is only about sixty-five miles wide, very shallow, and dotted with islands. The other gap between these masses is somewhat wider, but still not so great as the shortest distance between South America and Africa. Thus the arctic region is almost encircled by land and itself contains much land. The earth's surface is really one huge northern land mass with three southward projections, namely America, Africa and the East Indian Islands, including Australia. Antarctica is a small disconnected mass at the other end. A species or group of species originating in, or getting into, the far north could, as far as the present configuration of the continents goes, populate the earth and have solid ground under its feet most of the way.

Probably one of the main reasons for the little consideration which students of geographic distribution have given to this route of dispersal is the present climatic conditions in the far north. It seems to have been easier to imagine the ocean's bottom heaved up between Africa and South America than to conceive of a different climate in the northern regions. Yet we have definite and incontrovertible evidence of mild arctic climate in at least several geologic periods, while the only moderately strong evidence of a bridge across the Atlantic, for example, is the presence of related or identical forms on the opposite shores.

Suppose two of us are known to have been together on Broadway, but now one of us is in eastern Connecticut and the other at the eastern end of Long Island. One theory might be that we traveled together to the eastern end of Connecticut and then, while one of us stayed there, the other crossed the Sound in some way or other, even though we could not swim that far, there was no regular boat service, and the only evidence of a bridge having been built and then destroyed is that one of us is in eastern Connecticut and the other in eastern Long Island. It would seem to me more probable that we parted company in Manhattan and, while one of us crossed by a known bridge to Long Island and then had good going along the southern shore of the Sound, the other crossed known bridges and travelled through Connecticut along the northern shore of the Sound. This seems to be the way the respective theories concerning the biogeographical relationships of South America, Africa and Australia stand except that we have some other facts. To continue the comparison: although there may be no direct evidence as how one of us came to be in

eastern Connecticut and the other at the eastern end of Long Island, yet it is known that other people have left New York and gotten to these two places without crossing the eastern end of the Sound and, furthermore (to make the comparison more accurate), we must put in as a part of the argument that no one was ever actually known to cross directly from eastern Connecticut to Long Island or *vice versa*. In the face of such evidence he would be rash indeed who would hold to the first theory as to our movements after we left Broadway.

Now I know very little about geology and still less about fossil mammals, but I am willing to take on faith the conclusions of well-accredited students of these subjects if the conclusions seem to have been reached by logical deduction from reasonable premises and if the assertions of fact are not too widely different from my recollection of the assertions of fact made by students who have reached other conclusions from what seem to me to be unreasonable premises. It would be out of place here to give the details of Matthew's analysis of vertebrate, especially mammalian, paleontology. He takes up group after group and outlines their fossil record showing that they

accord fully and in detail with the principles[5] here set forth, and to be impossible of explanation except upon the theory of permanence of the ocean basins during the Cenozoic era. While the prominence of the Holarctic region as a center of dispersal is ascribed to its central position and the greater area, some evidence is given to show that climate is also a factor in the greater progressiveness of the northern, since it is also noticeable in the southern as compared with tropical faunæ.

The distribution of the Reptilia appears to be in conformity with the principles here outlined, and extends their application to the Mesozoic era. The distribution of birds and fishes and of invertebrates and plants is probably in accord with the same general principles, modified by differences in methods of dispersal. The opposing conclusions that have been drawn from the distribution of these groups are believed to be due to an incorrect interpretation of the evidence. A few instances, which have been prominently used to support opposing conclusions, are analyzed and shown to conform to the conclusions above set forth, if interpreted upon similar lines as the data for mammalian distribution.

As an example of the widely divergent conclusions which may be drawn from the same data concerning present-day distribution

[5] Permanency of the abyssal oceans; climatic cycles from extremes of cold or arid zonar climates culminating in glacial epochs, to the extremes of warm, humid, uniform climates, associated with cycles of moderate elevation and submergence, respectively, of continents; the cyclic development of progressive groups of animals in the great northern land mass and their dispersal southward as the result of these climatic changes.

I take the liberty of referring to my paper on the spiders of the Greater Antilles.[6] On pages 139 and 140 it is shown that sixteen Antillean genera, selected in a rather random fashion, are found to-day in regions connected by a certain hypothetical system of trans-oceanic bridges and not elsewhere. In some cases there appears to be almost no specific difference, even, between spiders whose present range is far from continuous. In fact, this set of data and other instances mentioned in that paper seem to me to furnish as strong evidence for such bridges as is given by the recent distribution of any one group of organisms, and yet I felt that this was not at all the explanation. It seemed more reasonable to believe that spiders had dispersed by the way of Holarctica on land masses which were practically the same as they are now and that the present discontinuous distribution of the ancient types is brought about by the fact that they are merely relicts which are now found far separated from each other.

Entomologists will at once think of a number of species which have reached the United States in historic times from Mexico and many will use these in a contention that much of our fauna has been derived from the south. I believe that the movement has been largely the other way and that the "southern element" of our fauna is largely made up of those things which have dropped behind in the general southern movement. As in any stream there are back eddies, so in the stream of dispersal we must expect back eddies (especially when man makes a channel as he did when he planted a large number of potatoes up to the former habitat of the Colorado potato beetle or grew great quantities of corn and other cereals for the chinch bug) but the eddies do not indicate the direction of the main stream. In their progress toward the equatorial region the streams of dispersal leave pools here and there—the stranded relicts of an ancient fauna. There are doubtless numerous swirls and back currents, while near their mouths these streams of dispersal may be much subject to "tides" due to minor, *i. e.*, measured by centuries, fluctuations of climate, but their general movement is, nevertheless, from the poles toward the equator. Since the northern polar regions have the larger land masses and better facilities for such dispersal-stream flow, the larger movements have been from the north, but simliar, though smaller, currents are to be expected in the southern hemisphere.

FRANK E. LUTZ

AMERICAN MUSEUM OF NATURAL HISTORY

[6] Lutz, F. E., 1915. "List of Greater Antillean Spiders with Notes on their Distribution." *Annals*, N. Y. Acad. Sciences, XXVI, pp. 71–148.

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THE
AMERICAN NATURALIST

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SEX CONTROL AND KNOWN CORRELATIONS IN PIGEONS[1]

Dr. OSCAR RIDDLE

Station for Experimental Evolution, Cold Spring Harbor, L. I., N. Y.

When one nowadays states that he has obtained a real control—a reversal—of the development of sex, he can feel assured that his biological audience demands a very large volume of rigid proofs. The first reason for this large requirement is, as you well know, that the assertion of sex control has been often made, and that in most of these cases the data have proved disappointing; inadequate in one or another respect. A second reason for present widespread skepticism as to even the possibility of a real control of sex-development centers in the now well-demonstrated fact that in some groups of animals, the male—and in other cases the female—produces sex cells of two kinds when these are considered from the standpoint of their chromosomal numbers or characteristics; and further that each of these two groups of germs *normally* gives rise to organisms of the sex corresponding to the chromosomal constitution of these germs. Moreover, certain linkage phenomena observed in breeding such forms, unquestionably show themselves to be *normally* associated with these same chromosomal differences.

But the experimentalist has learned through some pre-

[1] Paper read before the American Society of Naturalists, Columbus, Ohio, December 30, 1915.

vious contests with ideas of fixity and causality that when *normal* structural correlations have been demonstrated in the field of development, *nothing* has been decided as to *causality* and *inflexibility;* indeed it is commonly at such a point that experiment applies the pressure of *new* or *unusual* conditions and makes an approach toward learning the nature of a phenomenon, by forcing the latter to break from its normal correlations, and disclose something of its real nature through its versatility—through its own capacity to shift from response to one set of conditions, to response to another set of conditions. Laws of causation, in the field of development, are not to be deduced from studies concerning the normal associations of the *structures* of the cell; they may be approached through demonstrations of the versatility and responsiveness under pressure of those *processes* native to living matter.

We have stated that when sex is controlled an audience like this will demand a volume of proof. It is clear that the time limits here do not admit so extensive a presentation. I should like to note here, however, that Professor Whitman's complete studies on sex in doves and pigeons have been prepared for publication, and for several months have been in the hands of the publisher. The results of my own studies of the past five years designed to test the reality of the sex-control, and the nature of sex, as exhibited in these forms, will already doubtless fill another volume. And, since the last volume of the posthumous works of Professor Whitman is nearing completion, I can promise that it will not be long before the work of preparing my own results for publication will be begun. Only when all of these data are fully available to you, may we expect a judgment as to whether the evidence for our thesis to-day is adequate. It is possible to give here, within the time limits, only an outline of the *kinds of study* which have yielded evidence on the question of sex-control in pigeons.

These studies were begun, and carried on for many years by Professor Whitman. He obtained indispu-

tably—a profound modification of the sex-ratio, and identified in a general way the factors associated with the modified ratios. Whether the modified ratios signified a real control—a reversal—of sex could not at that time be definitely decided. It was to help in making a decision as to whether the changed sex-ratios signified a real—or only an apparent—reversal of sex that I proposed in the winter of 1908–9 to carry out some chemical studies on the ova of the doves and pigeons which in Whitman's hands were yielding these striking sex ratios. The methods for the quantitative and qualitative analysis, of the very small samples to be used, were developed, and these were tested during 1909–10 on considerable numbers of the larger ova of jungle fowls and domestic fowls. Since April, 1911, I have carried on this and other lines of study to determine if possible whether the changed ratios observed by Whitman involve a real reversal of sex; this work is being actively continued.

Whitman showed that "width of cross" in doves and pigeons is of first importance in determining sex ratios and that the wider the cross the higher is the proportion of males. Family crosses produce—in practically all matings—only male offspring. Generic crosses produce from their "stronger" germs—those of spring and early summer—nearly all males. If, however, the birds of such a generic cross be made to "overwork at egg-production"—that is if their eggs are taken from them as soon as laid and given to other birds for incubation—then the same parents which in the spring threw all or nearly all male offspring may be made to produce all, or nearly all, female offspring in late summer and autumn. At the extreme end of the season eggs capable of little, then of no development, are often found in such a series. As the birds of such a mating grow older the time of appearance of females, and of eggs incapable of full development, is reached earlier and earlier in the summer or spring.

In the case of a number of hybrids Whitman showed that *color* is also affected by this pressure of reproduc-

tive overwork and season. White color could be obtained from the later, "weaker" germs, though this color did not appear in birds from the "stronger" germs of the earlier season. And further, that white, or whitened, "mutants" from pure breds were derived almost or quite exclusively from those conditions which produce "weakened germs." Among such conditions are late season and overwork, inbreeding and great extremes of age—either very old or very young. This brief outline of Whitman's findings on sex is perhaps a more adequate, and more accurate, one than I was able to give to one of the societies represented here when I had only begun the examination of this data in 1911. Two brief summaries given on the chart (not given here) will assist in obtaining a picture of the nature of the results. I may add that by very strongly "overworking" females of some species—overworking them more strongly than Whitman did—I have been able to obtain a high predominance of females during autumn from a cross merely of *specific*[2] value. This result is illustrated by Chart II, though the matings there exhibited were prepared for the primary purpose of illustrating results in the study of size. It will be noted in the chart that parents overworked in a previous year throw a high proportion of females during the whole of the succeeding year, and most markedly in late autumn. In this mating the ratio at the end of the season is 14 females to 1 male; in the other (not previously overworked) there was an excess of females only after overwork—during the latter half of 1914 (7♂:10♀); and in the year following this overwork there were 21 or more females, to 11 or fewer males. Such data are not exceptional; they coincide with the usual.

Now, in the generic crosses which give all, or nearly all, males at the beginning of the season and all, or nearly all, females in the autumn what is happening?—true sex reversal? or is it selective fertilization, differential mat-

[2] Some data from pure breds (pure species) mated to their own kind show also this predominance of females from late autumn under extreme overwork; such predominance is here probably less pronounced than in the case of the crosses.

uration or a selective elimination of ova in the ovary? This was from the first the whole of our own problem. We have had no other, nor have we now, except in so far as the entire question of the *nature* of sex—in germ and adult—is concerned.

Our method has been to study the eggs, progeny and parents of such series as show this seasonal "reversal of the dominance" of sex from as many different angles as possible. The result till now is that we have learned some ten kinds of facts concerning the germs, or the prospective value of the germs, which issue from such a series. Let us note that these ten lines of correlated fact do not relate merely to a "normal" state of the germs, but have to do with measurable changes which occur when ova are subjected to the stress of parental reproductive overwork, which as Whitman has shown is accompanied by a shifting from male-production to female-production during the progress of the season. The diagrams of chart I will assist in making clear the nature and significance of the several correlations. The solid lines indicate a *double* correlation, *i. e.*, for both season and egg of clutch; the broken lines represent correlations established thus far for only one of these.

The generic cross that has been most fully studied involves *Turtur orientalis*—the Japanese turtle dove, and *Streptopelia alba*—the white ring dove. These species together with their reciprocal hybrids are shown (photographed) in another chart (not given here). Some data for egg size, and for sex-differences in the adult size of the several forms concerned—parents and reciprocal hybrids—are also given in that chart.

The first correlation that we have established for this series results from a study of the size of ova—*i. e.*, of yolks freed from shell and albumen. The result clearly establishes the fact that the yolks of late summer and autumn—those that produce mostly, or all, females—are larger than the yolks produced in the spring which give rise to males. And there is no jump from the one size to the other, but what may be better described as a

gradual enlargement. This fact is represented diagrammatically on chart I, and some of the actual figures may be had from the charts dealing with size of offspring (II), with analyses (III), and with calorimetry (IV).

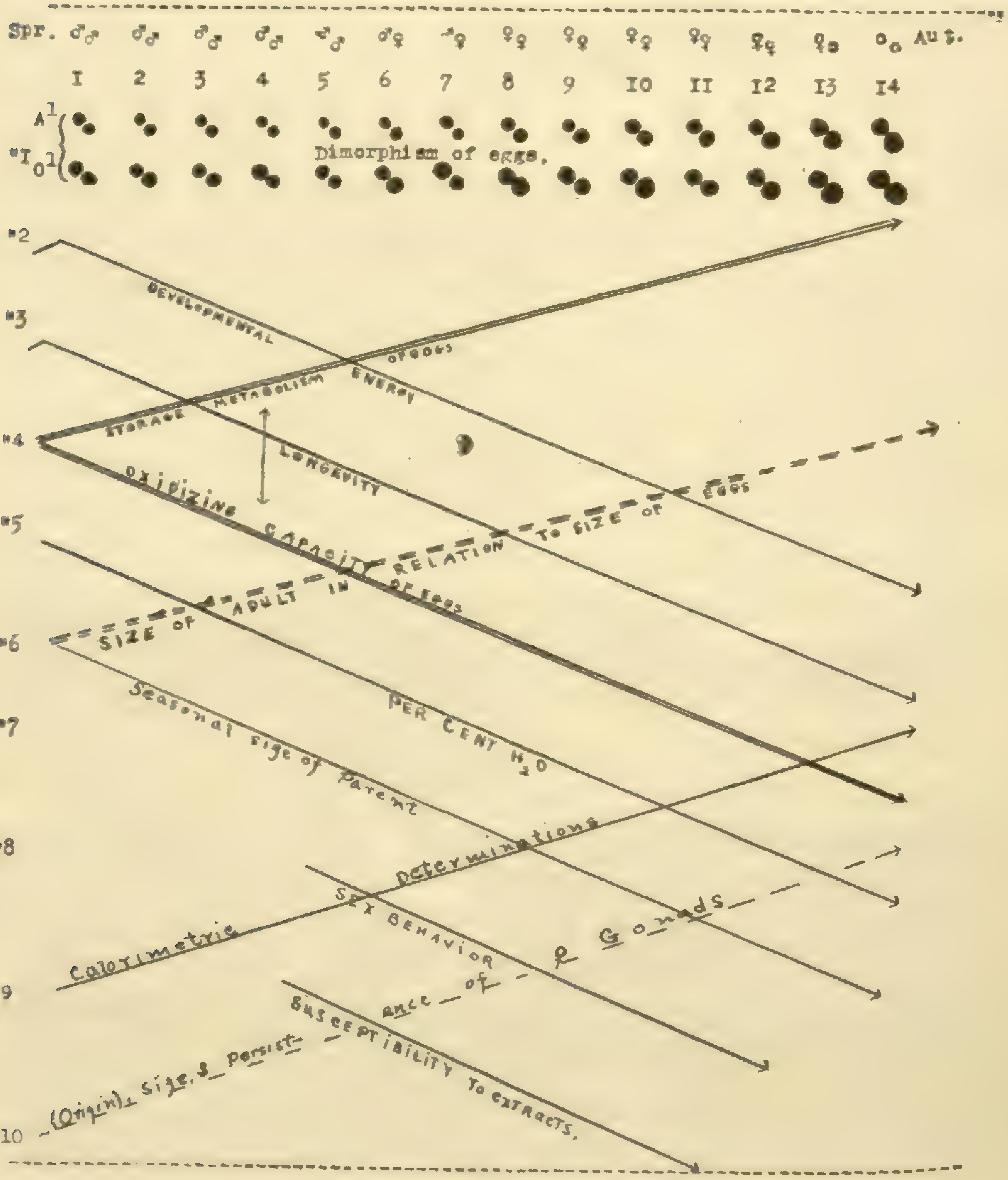

CHART I

COMPARATIVE SIZE OF EGGS OF ALBA (*a*) AND ORIENTALIS (*o*).

At the same time that this seasonal increase in size of yolks was learned, it also became evident that the eggs of doves and pigeons are *dimorphic*: That the two eggs forming the pigeon's clutch bear usually—there are exceptions—a smaller yolk in the first, and a larger yolk in the second. Since Whitman had already shown that

in the pure wild species with which he worked, males predominate in hatches from first eggs of the clutch, and females predominate in hatches from second eggs of the clutch, it became evident that the male-producing yolk is smaller—both in relation to season, and to egg of clutch, than is the female-producing yolk. Corresponding to the fact (commonly obtained from matings of individuals of the same species) that two males or two females may sometimes arise from the same clutch, we have found that a similar number of pairs of yolks of these forms are equal in size; and too that such pairs may be either large or small. The charts just referred to may be consulted in this connection. We have previously noted (1911, 1912) that in eggs laid by hybrids neither sex nor yolk-size bears the above described relations to the order of eggs in the clutch.

Still a third situation has yielded positive evidence that the smaller yolks are male-producing and the larger yolks female-producing—namely that in respect to age. It has already been mentioned that Whitman learned that the females which were "overworked" tended, when older, to begin the production of females at earlier and earlier stages of the season. Now a comparison of the size of yolks derived from younger and from older birds has conclusively shown smaller eggs for mature but younger birds, as compared with the old birds (see Chart 2). In scores of individual cases the yolk-size has now been followed from youth, and comparative youth, to old age.

In even a fourth situation it has been possible to test the relation of yolk-size to sex. Breeding data show that from the very first egg in life, and the very first egg produced after a long period of rest or inactivity, more frequently produce a *female* than do the first eggs of succeeding pairs, or clutches. Our studies on the size of such yolks show a wholly similar reversal of order of size of the two eggs of the very first clutches; the size reversals here being more frequent than in the succeeding clutches (see Chart 4).

CHART II

BREEDING RECORDS—1914

♀ *St. risoria 641 (old); 1913 = 42 eggs*

Series 1

♀ A1	1/1	White 140
♀ A2	1/3	White dead 2/3
1st (4) = 2.066 g.		2d (4) = 2.243 g.
H1	4/4	Inf. yolk = 1.995 g.
H2	4/6	Inf. yolk = 2.105 g.
♀ I1	4/12 }	{ White killed 4/29
♀ I2	4/14 }	{ White 158 (?2)
♂ J1	4/21	Dark killed 2/25
♀ J2	4/23	White 158
♀ K1	4/29	White 147
♀ K2	5/1	White 151
L1	5/9	Broken
L2	5/11	Dark
♂ M1	5/18	Dark 161 (?1)
♀ M2	5/20	White 163
♀ N1	5/30	White 150
♀ N2	6/1	White killed with ext.
♂ O1	6/7	Dark 150
♀ O2	6/9	White 150
♂ P1	8/18	Dark 149
P2	6/20	Broken
♀ Q1	6/28	White 143
♀ Q2	6/30	White 137
♀ R1	7/4	White 154
♂ R2	7/6	Dark 162
? ♂ S1	7/12	Dark dead 7/29
♀ S2	7/14	White dead 7/31
♀ T1	7/20	White 140
♂ T2	7/22	Dark 164
♀ U1	7/28	White 144
♀ U2	7/30	White 151
♀ V1	8/14	White 155
♂ V2	8/16	Dark 169
♀ W1	8/22	White 152
W2	8/24	Soft at pole
♀ X1	8/30	White 161
♀ X2	9/1	White 145
♂ Y1	9/9	Dark 161
♀ Y2	9/11	White killed
♀ Z1	9/18	White ——
♀ Z2	9/20	White dead 10/26
♀ AA1	9/26	White 141
♀ AA2	9/28	White 146
♀ BB1	10/7	White 150
♀ BB2	10/9	White 144
? ♀ CC1	10/17	Dark dead 11/8
♀ CC2	10/19	White dead 11/10
♀ DD1 }	{ 10/26	White 130 (?1)
♀ DD2 }	{ 10/28	White 162 (?2)
♂ EE1	11/6	Dark 152
♀ EE2	11/8	White 143
♀ FF1	11/16	White 166
FF2	11/18	Broken
♀ GG	11/26	White 150

1st 16 = 5♂ : 11♀ 2d 15 = 5♂ : 10♀ last 15 = 1♂ : 14♀

The relation of the order of the eggs in the clutch to the prospective sex of the offspring is an important point, and we wish here to make this situation clear, since it seems that two rather brief statements made in 1911 and 1912, before the Society of Zoologists, have not been understood by all.

From the time of Aristotle to the present year there

CHART II—*Continued*

BREEDING RECORDS—1914

♀ *St. risoria 647 (young); 1913 = 18 eggs*

Series 2

C1	2/8	Inf. yolk = 1.445 g.
C2	2/10	Broken
♂D1	3/5	Dark embr.
♀D2	3/7	White
♂E1	3/19	Dark 167
♂E2	3/21	Dark 180
♀F1	3/29	White 154
♂F2	3/31	Dark 190
♂G1	4/8	Dark killed 5/6
♀G2	4/10	White killed 5/3
♀H1	4/16	White 153
♀H2	4/18	White 153
♂I1	4/25	Dark 169
♀I2	4/27	White 154
J1	5/5	3-da. embr. killed
J2	5/7	3-da. embr. killed
♂K1	5/14	Dark 169
♀K2	5/16	White 158
♂L1	5/25	Dark 179
♀L2	5/27	White 164
♂M1	6/3	Dark 169
♀M2	6/5	White 11 da. emb.
♂N1	6/13	Dark 165
♀N2	6/15	White 150
♂O1	6/22	Dark killed 7/13
O2	6/24	Broken
♀P1	7/1	White 150
♀P2	7/3	White 15 da. emb.
♀Q1	7/9	White 148
♂Q2	7/11	Dark 164
♀R1	7/22	White 152
♂R2	7/24	Dark 172
S1	8/3	White 13 da. emb.
S2	8/5	Broken 3 da. emb.
♂T1	8/12	Dark 174
♀T2	8/14	White 164
U	8/20	yolk = 1.490 g.
V1	9/6	"Blood circle"
♂V2	9/8	Dark 170
?W1	9/19	Dark dead 10/16
♀W2	9/21	White dead 10/14
♂X1	9/30	Dark dead 10/19
♀X2	10/2	White 145
Y1	10/29	Inf. yolk = 1.845
♀Y2	10/31	White 15 da. embr.
Z1	12/27	No dev. yolk = 1.870 g.
Z2	12/29	No dev. yolk = 1.925 g.

♀641 = (170) (♂170)
♂'s (5) from 1st = 155 ♀'s (13) = 149
♂'s (3) from 2d = 165 ♀'s (11) = 150

♀647 = (166) (♂165)
♂'s (7) from 1st = 170 ♀'s (5) = 151
♂'s (5) from 2d = 175 ♀'s (6) = 158

1st 17 = 9♂ : 8♀ 2d 17 = 7♂ : 10♀ *1915* = 11 dark : 21 white

have appeared statements concerning a predominance—or a lack of predominance—of males from the first egg and of females from the second egg of the pigeon's clutch. It is unnecessary to outline these divergent reports. It is only necessary to point out the reason for discordance; though the reason we had thought to be quite obvious since 1911. The statements hitherto made have all been based on a general statistical method, which is a wholly

CHART III

SUMMARY OF PARALLEL BREEDING AND CHEMICAL STUDIES ON THE EGGS OF ♀ *T. orientalis* No. 500X *St. alba* No. 410 FOR THE YEAR 1912

Date	An'l's or Inc.	Wt. of Yolk	Result						
			Alc. Soluble	Phosphatids	Protein	Ext.	Ash	H_2O	Energy Total
4/13			Broken when found						
4/15			Broken when found						
5/26	159	2.330	72.65	18.32	25.44	5.28	4.85	57.01	7,405
5/28	160	2.660	72.45	17.54	25.63	5.25	2.62	54.82	8,990
6/7	Inc.	....	Only one egg laid						Dark ♂
6/15	Inc.	....	....	....	....	....	....	....	Dark ♂
6/17	Inc.	....	"Very large egg"			....	....	....	White ♀
6/24	Inc.	....	....	....	....	....	....	....	No. dev.
6/26	Inc.	....	....	....	....	....	....	....	Dark ♂
7/3	186	2.026	72.21	16.49	26.00	3.63	2.43	56.05	6,714
7/5	187	2.330	72.27	19.18	26.55	3.75	1.93	55.22	7,881
7/15	Inc.	....	....	....	....	....	....	....	Dark ♂
7/17	Inc.	....	....	....	....	....	....	....	Dark ♂
7/23	192	2.422	72.42	17.82	25.88	3.82	1.80	55.84	8,061
7/25	193	2.720	72.45	18.88	25.96	3.86	1.81	55.33	9,296
8/2	Inc.	....	....	....	....	....	....	....	Dark ♂
8/4	Inc.	....	....	....	....	....	....	....	Dark ♂
8/13	Inc.	....	....	....	....	....	....	....	No. dev.
8/15	Inc.	....	....	....	....	....	....	....	Dark ♂
8/23	Inc.	....	....	....	....	....	....	....	No dev.
8/25	Inc.	....	....	....	....	....	....	....	White ♀
9/15	Inc.	....	....	....	....	....	....	....	White ♀
9/17	Inc.	....	....	....	....	....	....	....	White ♀
11/29	259	2.700	73.17	21.40	25.23*	....	....	55.52	9,323
12/1	260	2.715	73.02	21.63	25.38*	....	....	55.39	9,383

* Calculated.

inadequate and useless one for a study of the problem. It is now clear that the method that would be valuable for this purpose must be a thoroughly *analytical* one. Whitman has properly analyzed this situation. He has shown that normally—*i. e.*, with effects of *crossing* eliminated—from the periods for the production of the strongest germs an undue proportion of *pairs* of eggs produce males; and from the opposite period there arise undue

CHART IV

STORED ENERGY OF EGGS (1914) OF *Streptopelia risoria* (558) AS DETERMINED BY THE BOMB CALORIMETER

No.	Date	Wt. Yolk	Energy	Per Cent. Diff.
665	A1 6/6	1.010[1]	3,358[1]	
666	A2 6/8	0.970	3,175	−5.8[2]
674	B1 6/19	0.855	2,807	
675	B2 6/21	1.000	3,245	+15.6
699	C1 7/14	1.145	3,815?	
700	C2 7/16	1.463	5,008	+31.3?
728	D 8/30	1.395	4,812	
——	E 9/9 or 10 soft shell, broken			
...	F1 10/17	" " "		
...	F2 10/19	" " "		
770	G1 11/6	1.440	4,837 (?)	
771	G2 11/8	1.720	5,797	+19.8 ?
774	H1 11/20	1.590+sl. loss	4,906+	
775	H2 11/22	1.780	6,015	+22.6−
776	I1 12/1	1.640	5,614	
777	I2 12/3	1.820	6,255	+11.4
781	J1 12/12	1.535	5,302	
782	J2 12/14	1.690	5,601	+5.6
791	K1 12/23	1.485	5,266 (?)	
792	K2 12/25	1.718	5,880	+11.7 ?

[1] This egg was not only the first laid during season, but first during life of this bird.

[2] The percentage differences are based upon a value of 100 per cent. for the smaller egg of the pair.

numbers of pairs of eggs that produce females. To lump these all together and to count the number of males arising from first, and females from second eggs is plainly to cover up or to lose the significance of the intervening pairs of eggs which bear the significant data. Again, many matings, because of exceptional strength or of weakness, will yield a considerable total predominance of males or of females, and the statistical method lumps all these and others without thought or care of the cancellations and unsatisfied cancels involved; all of which as easily contributes to a *smoothing* of the results, as it does to a *smothering* of them.

But Whitman has also shown that not only is the *method* previously employed at fault, but that, much more important still, the *material* used—in probably all of those cases in which no correspondence of sex to the

order of the eggs of the clutch was found, and where the worker has thought it worth while to mention the *kind* of birds studied—such material has been wholly unsuitable to leading to a decision. That is to say, the "pigeons" used in these cases were one or another of the 150 *mongrels* collectively known as domestic pigeons. One of the clearest points of our present knowledge of the relation of sex to egg of clutch is that the normal relations are lost *immediately upon hybridization—i. e.*, in passing from the *pure state of the species*. The countless degradations and crossings suffered by the various domesticated breeds since their existence as a pure species, is therefore a sufficient index of the suitability of this material for a study of this subject. Whitman demonstrated the predominance of males from the first, and of females from the second egg of the clutch when pure species mated with pure species produce the eggs, and also the random distribution of the sexes from the eggs of hybrids. And as early as 1911 and 1912 I demonstrated charts and lantern slides which showed that the size of the yolks from pure species showed with considerable uniformity a smaller first, and a larger second yolk; and further, that this regularity breaks down at once and completely in hybrids.[3]

Let us now note the conclusions which follow upon the demonstrated dimorphism of the ova[4] in the pigeons, when this is reviewed in the light of breeding data on these forms and in connection with the demonstrated relationships of size of yolk to sex—relationships which are continued even under the pressure brought by overwork, season, and age.

It becomes clear, first of all, that a selective fertilization by one kind of sperm is quite impossible—the sex

[3] Note that in Chart 2, already referred to, where the eggs are produced by the female cage or blond ring dove—in which *purity* of the species is often doubtful—that a predominance of males from first, and of females from the second egg of the clutch is indicated in both series. In series I, where the two sexes arise from a single clutch, the first egg gave rise to the male in 6 (or 7?) cases; to a female in 3 cases. In series II the first egg yielded males in 9 (or ?10) cases; females in only three cases.

[4] Yolk size has now been accurately determined in about 10,000 cases.

differential residing in two kinds of eggs and not in the sperm. We may here recall that previous to our own studies, breeding data obtained from other birds had indicated that in the birds the sexually dimorphic germs are borne by the female—or to use Mendelian terms, that the female bird is heterozygous for sex.

The second conclusion that must be drawn is that a selective elimination of ova in the ovary does not occur during "overwork," while mated to a mate of another genus, nor otherwise, since the two kinds of ova are—from their size relations—positively known to present themselves under these, and under all the conditions which have been studied. In other words, the generic cross, which produces all or nearly all males in the spring, and all or nearly all females in the autumn, is utilizing in the spring a number of female-producing ova for the production of males, and in the later season is utilizing for the production of females ova one half of which had initial inclinations for the production of males. Note too that the evidence for the continued production during the season of ova of two kinds as regards sex does not rest alone on our knowledge of the dimorphic ova. For, from breeding data we learn that if the *same female* which threw all males in the spring and all females in the autumn, had been mated to one of her *own* species, then both males and females would certainly have appeared at all seasons, and largely or wholly in relation to the order of the eggs of the clutch, with but slighter effects of season to be noted. If the overwork were extreme, a predominance of females in late autumn might be expected; but in the earlier season the sexes would surely be found in nearly equal numbers. Several of the correlations soon to be mentioned, moreover, further attest that ova of two grades—in respect to sex—are produced throughout the year.

The data thus far examined exclude the possibilities of accounting for the observed sex-ratios of the generic cross on the basis of a selective action of the sperm, or of a selective elimination of ova in the ovary. What light do these data shed on the possibility of accounting

for the seasonal difference in sex-production on the basis of a differential maturation? The fact that the sperm is present in the pigeon's egg during the whole of the second maturation division may properly raise this question. On this point we must say that the particular data we have just been citing are perhaps not entirely conclusive; these data alone, however, offer the following significant points for consideration: To account for the observed sex ratios of the *generic cross* the maturation would have to be definitely differential in (1) the elimination of an X chromosome[5] during the spring from one half of the ova, and the retention of this same X in the homologous[6] eggs of the autumn. (2) The elimination of a Y chromosome from the other half of the eggs laid during the autumn, and the retention of all these same Y's in homologous eggs of the spring; and (3) all other chromosomes than the sex chromosomes must display no such thing as seasonal preferences for "staying" or for "going," since every observable character of the hybrids betrays the presence of both of the parental genera. This is not all, but let us pause at this point to note that even if the sex chromosomes were here capable of such wholly unknown and almost unthinkable behavior, that they have—after all—in this case wholly lost the *initiative* in governing sex, since it is the *place* in the season and the *degree* of the *pressure* of the *overwork* that has been shown to prescribe the sex of the offspring; and further, the correlations of size, water content, energy storage, etc., which we have proved to exist throughout the whole season—these correlations are all established *prior to* the formation of even the first polar body; this latter being formed only at the time of ovulation, and the second polar body forming 1 to 1½ hours after the entrance of the egg into the oviduct.

If, however, we were inclined to set no bounds to the

[5] The chromosome situation in the germ cells of female doves and pigeons is as yet quite unknown. But whatever it may be, our statement illustrates the difficulties of a chromosome theory in the cases under consideration. We make use of a familiar case in which XY germs are male-producers, and XX germs female producers.

[6] *I. e.*, in eggs of identical (original) chromosomal constitution.

marvels of selective power that may be exhibited by the sex chromosomes, and to feel that even the above difficult formula remains for them a possibility, we may refer to the decisive data obtained in studies on the sex behavior of the birds which are hatched from such a sex-controlled series. We shall there see that those data differentiate *several grades of females*. Some are quite nearly males, —though they lay eggs. Is it too hazardous to suggest that in one and the same egg the Y could hardly have "gone out" to allow the egg to develop into a female, and yet have "stayed in" in order to deliver the relative masculinity that we easily detect and measure? If sex is directly the creature of a sex chromosome, the sex situation found in some of my female doves requires that the male-producing chromosome be eliminated from, and retained in, one and the same egg! The only alternative that it is within my power to imagine is that in addition to the selective elimination of the Y's during autumn, there be further postulated a gradual fractional elimination of parts of this chromosome, larger and larger parts being eliminated during the progress of the season. Or, that the reverse of this occurs, namely that the Y, during the progress of the season, *gradually adds* something of X quality to itself, finally becoming more X than Y. For those who would value this interpretation I have no evidence or word of contradiction. The fact must always remain that our procedures have not only produced male and female from ova of opposed initial tendency—largely under control—but that several *grades of intermediate sex* have also been produced.[7]

[7] Three previous publications, besides several addresses before the American Society of Zoologists and elsewhere, have clearly stated this result. The publications now two years since, and the citations are as follows: (*a*) *Carnegie Year Book*, No. 12, 1913 (p. 322), Report of Year's Work. "The results strongly indicate that the hereditary basis of sex (and, therefore, probably all characters) is a quantitative, graduated thing; not qualitative and alternative as rather generally believed." (*b*) *Science*, N. S., Vol. 39, No. 1003, Mar., 1914 (p. 440), "A Quantitative Basis of Sex as Indicated by the Sex Behavior of Doves from a Sex Controlled Series." "These . . . results together with our very abundant data on the storage metabolism of the ova of these forms, and the initial fact of sex-control itself, strongly

We shall be able presently to note more closely the conclusive facts as to the matter of a differential maturation. Continuing our examination of the further data which we know correlate with this sort of a sex-series we shall meet with additional and other kinds of facts which lead toward a constructive view of the nature and basis of sex; facts immediate and specific concerning the measured powers or capacities of these series of ova which present us the sort of sex-series in question—facts which reveal sex in quantitative terms.

Correlations marked (2) and (3) on Chart 1 were first noted by Professor Whitman. I have been able every year to find many confirmations of his conclusions.

The curve for "Developmental Energy" on the chart indicates a progressive seasonal decrease of this capacity in the fertilized eggs; a decrease from spring to autumn. Now the evidence is unquestionable for the lowest part of the curve—the autumn. In general, least development proceeds from the last eggs of the season. These are the *largest* eggs of the year. There is also less development in the second eggs of the clutch. These are the larger of the clutch. It is thus seen that the larger the yolks the less "developmental energy" possessed by them.

The "Length of Life" of the several offspring of such a sex-series tells again of an advantage possessed by the earlier hatched birds, and of a more limited life-term affixed to the later hatches. It is further probable that within the group of clutches giving rise to females only, a longer average life-term falls to those who hatched from the first egg of the clutch, than to those arising from the second. Here, then, as in correlation no. (2) the smaller eggs of clutch and season are the eggs pro-

indicate that the basis of sex is a fluid, reversible process; that the basis of adult sexual difference is a *quantitative* rather than a qualitative thing." (*e*) *Bulletin of the American Academy of Medicine*, Vol. 15, No. 5 (October, 1914) (pp. 265–285), "The Determination of Sex and Its Experimental Control." "The sum of these results, together with the initial fact of sex control itself, practically prove that the basis of sex is a fluid, reversible process, that the basis of adult sexual difference is a *quantitative* rather than a *qualitative* thing (p. 277)." etc., etc.

ductive of "strength." The larger eggs both of clutch and season more often display "weakness." And in passing we might note that by the procedures involved in these sex-series it is possible to graduate the fatal dosage, and in great measure to predict which of particular germs must come to an end first.

The fourth kind of fact pertaining to the eggs of this series, proceeds from the results of more than 800 chemical analyses of individual eggs. The results of earlier studies of this nature were described in 1911[8] and 1912 more fully than time limits will here permit; but the nature of these results can be noted with the help of Chart 3. It will be observed that not only does the size of the egg increase with its later position in the series, *i. e.*, with lateness of season, but the percentage of energy-yielding or stored materials increases as much as, or possibly more than, is indicated by the size—or net weight—of the yolk.

The importance, for our present purpose, of the results of these analyses is that they conclusively show (1) that the male-producing egg of the spring is an egg that stores less material than does the female-producing egg of the autumn. (2) That the male-producing egg of the clutch *stores* less material than does its female-producing mate. (3) That the eggs of old females *store* more materials, and—as has been noted—yield a higher percentage of females, than do birds not old. Therefore, it becomes evident that the egg of female-producing tendency is one whose storage metabolism is high, as compared with eggs of male-producing tendency. The analyses show that during the season successive clutches present higher and higher storage, *i. e.*, the earlier clutches store less—are more male-like; the later ones all store more—are more female-like; and as we have seen, the eggs of the low storage period give rise to males, those of the high storage period produce females. Here we obtain a close view of that upon which sex difference rests. Un-

[8] Papers read before the American Society of Zoologists. For abstract, see *Science*, N. S., Vol. 35, pp. 462–463, March 22, 1912.

mistakably, less storage and high storage pertain respectively to the male- and female-producing germs. Unmistakably, our procedure—connected with generic cross, season and overwork—delivers males from the smaller storages in the earlier eggs. Unmistakably, these procedures raise the storage in all of the later eggs, and unfailingly we then find that these eggs yield only, or almost exclusively, females. And if we eliminate the factor of wide—or generic—cross and mate the female with one of her own species, then we see that the production of males and females coincides from the first with two sizes of eggs in the clutch—males from the smaller first, female from the larger second. Only after overwork and season have raised the storage value of the eggs, is this situation, in such a mating, seriously disturbed. And the disturbance—associated with an increase in the storage metabolism of all the eggs,—delivers, as before, an excess of female offspring.

The progressive *increase in storage capacity* of the eggs during the season—under overwork—is to be interpreted as a *decrease in the oxidizing capacity* of these same eggs. Living cells in general dispose of ingested food material by storing it, or by burning it. The products of the oxidation are removable and do not serve to increase the bulk of the cell. Likewise the low-storage capacity of the male-producing eggs as compared with the high-storage capacity of female-producing eggs is therefore an index of higher oxidizing capacity of the male-producing eggs as compared with the female-producing eggs.

The fifth correlation relates to the percentage of water in the eggs of spring and autumn, and in the two eggs of the clutch. These figures for one series of analyses are given on the chart (3) last examined. They show a higher water content for the eggs of the spring (male-producers) as compared with the eggs of autumn (female-producers); indeed, each pair of eggs from the first of the season onward has a slightly higher moisture value than the pair that follows it. The analyses further show a higher percentage of water in the first egg of the clutch

than in the second in all cases. If the results of my 800 analyses all ran as smoothly as do the 8 of this series there would be no doubt of a perfect correlation of high moisture values with small eggs, *i. e.*, with male-producing eggs —both small eggs of season, and small eggs of individual clutches. But the results are not thus uniform and smooth. There are some series which seem seriously to depart from the order noted above. These can not be discussed here. We can, however, record our own belief that the situation represented in the chart is, in the main, indicated by the moisture determinations.

Now the evidence that higher water values are associated with male-producing eggs, lower water values with female-producing eggs is of high importance in connection with our own generalization as to the basis of germinal sex-difference; and is further of much interest as being the means of demonstrating that in the—as I believe—several valid cases of sex-control now known, one thing in common has really been effected, this though the work has been carried out on a considerable variety of animals, and though the procedures have themselves been most various. The thing that seems to have been effected in all cases has been the *raising or lowering of the general metabolism of the treated germs.* If this conclusion be definitely established biology may congratulate itself that the further and complete analysis of this hereditary character lies near at hand; is open to definite and easy attack by methods already of demonstrated trustworthiness in this and other fields. And surely if such result is possible it is timely, now when the "box within box" revival has the sex character, like all others, dissociated from all *processes* that can be studied or measured, and associated with a *particle* so minute as hopelessly to defy all direct and functional investigation.

That higher water values in the tissues is associated in *development* with increased metabolism is a fact well established. We need cite here in reference to "tissue growth and repair" only the well-known fact of the higher water-content of embryonic tissues, and Minot's calcula-

tion that in a particular mammal 99 per cent. of growth power is lost before birth. In respect to "heat production" or the "basal metabolism" of embryo and adult the data for comparison are not extensive, but it too lends support to the view that this basal metabolism is higher in the young than in the adult. It may be added that Benedict and Emmes[9] have recently shown by very exact measurements that the basal metabolism of men is higher by about 6 per cent. than that of women.

If a higher metabolism exists in male-producing *germs*, and this is associated with higher water-content, as we concluded in 1911, it is easy to see why a number of procedures have since been shown to effect a control of the production of sex. In 1912 Miss King desiccated toads' eggs and obtained 87 per cent. of females. This was the converse of the earlier experiments of Hertwig, and of Kuschekewitch, who "over-ripened" frogs' eggs—a process during which they were found to *take up water*—and obtained, in the experiments of the latter author, as many as 100 per cent. of males. I think we can now see it was a shifting of the metabolism, through the agency of the water values, that produced the shifting of sex in the eggs of the frog and the toad.

More recently still, Whitney has effected a change in the sex of the offspring of the rotifer—*Hydatina*—a change from female- to male-production by means which he considers as serving to increase metabolism in the treated forms. Confirmation of Whitney's conclusion that it is a heightened metabolism that brings about male-production is now to be had in the result obtained by Dr. A. F. Shull[10] who finds that an *increased oxygen supply* leads toward an increased production of males in *Hydatina*. It now seems clear that a *heightened metabolism* in the Rotifers is the agency of increased male-production.

[9] Benedict, F. G., and Emmes, L. E., "A Comparison of the Basal Metabolism of Normal Men and Women," *Jour. of Biol. Chem.*, Vol. 20, No. 3, 1915.

[10] Advance abstract of a paper to be presented at these meetings, December 29, 1915.

The greater production of males in cattle—indicated by Thury, Russell, and several others—from eggs that have remained unfertilized for a period of hours, is almost certainly correlated with an increased water-content which these eggs obtain before fertilization. We do not know by direct observation that the ova of the cow takes up water from the fluids that it meets in the reproductive passages. We do know that this is true for the eggs of every amphibian, reptile and bird that has been investigated. Von der Stricht has, however, described phenomena in the yolk granules of the extra-ovarian egg on one mammal—the bat—which phenomena I am quite assured from my own earlier studies on the yolk spheres, definitely indicate that in this one mammal in which the data permit a judgment, the egg does take up water from the fluid that it meets in the Fallopian tube. There is good reason to believe that the changed sex-ratios of cattle can be associated with changes in the egg-metabolism effected through, or connected with, differential water values.

The important recent work of Baltzer convincingly shows the plastic, fluid, controllable and reversible nature of sex in *Bonellia*. And, it would be difficult to believe that the larva that attaches itself to the "rüssel" of an adult, then quickly and fully differentiates, and becomes a *male*, is not displaying a higher metabolism than is the larva that rests for long in the mud and sand, and after prolonged growth becomes a *female*. Baltzer's results deserve a much more extensive statement than can be given here.

Many points, too, in Geoffrey Smith's illuminating studies on sex in the spider crabs would seem to be in harmony with the view that the castrated males progressively lose their initial advantages of a higher metabolism, and that they then become more female-like as they approach the lower metabolic levels which are normal to the females. Though Smith, so far as I am aware, has not thus interpreted his results.

The point to these citations is that sex control, in the several various forms in which it has been accomplished,

has been accomplished fundamentally by the same means in all—a changed metabolism, in which a higher water-content of germ and higher metabolism for male-production, and lower water-content and decreased metabolism for female production, have been definitely shown to be associated in a number of instances. Whitman learned in pigeon hybridization an additional—an entirely different—*means* of accomplishing the *same end* of heightening the metabolism of the germ. And, this additional means definitely tends toward male-production. The wider the cross (within the limits of the "developmental compatibilities" of the germs) the greater the vigor and strength added *by the mere act of crossing*—and at the same time the more assuredly will such crosses produce males. Even the closely related varieties used in most Mendelian crosses have not failed to indicate the greater vigor of the heterozygote.

A sixth series of studies has been made on size of the parents and offspring concerned in these sex-controlled series. Seasonal and age fluctuations in the parents, and in both sexes of both parent species; size of offspring as related to their sex, to season, and to the egg of clutch, have been studied during three and one half years. We have found no subject that presents so many complications as does the matter of the size of offspring in this series. Only a single aspect of the matter will be treated here. The seasonal fluctuation in size of the parents used in the "overworked" or sex-controlled series is, however, a simple matter. Our results show—as indicated by the lower curve on the chart (1)—that such parents weigh most in winter and spring; least in the autumn, reaching a minimum in August and September. In other words, during the period when the female parent lays her largest eggs, she herself, and her consort, are smallest in size. I have had no charts prepared showing the seasonal curves for individual birds, but data for such curves in great number are available.

Now, the single word I wish to say on the relation of size in the offspring to the order of the eggs of the clutch,

and as affected by the procedure of overwork, may be more quickly said with the aid of the charts.

One chart (only Chart 2 is reproduced here) shows the weight average of each individual hatched during the year, from two simultaneous matings of *alba* × *risoria*. Series I is from an older pair, previously overworked; series II is from a younger pair, little—or not at all—previously overworked. It will be noted that series I is throwing large eggs, a predominance of females, and that the *size* of the offspring—even of the males—is prevailingly that of the *females* rather than the males of the parent species.[11] Series II is throwing smaller eggs, a nearly equal proportion of the sexes, except at the end of the season, and the size of the offspring is decidedly larger than in series I; and, in fact, approximates to the size of the *males* of the parent species. In both of these series it will be observed that size of offspring[12] is also correlated with the order of eggs in the clutch.

For series I, we have complete data for the year preceding and the year following the term covered by the chart. The weights for the former were: Av. for ♂'s 172 gr.; ♀'s 166 grams. For the succeeding year—early 1915—these weights are ♂'s 157 gr.; ♀'s 156 gr. Clearly, during the three-year period a change in size of offspring is progressively occurring; and the change runs from a size comparable to that of the males of the parent species, to a final size that is somewhat below that of the females of the parent species. The egg-size was known in this same series to have progressively and simultaneously changed from greater male-producing tendency to a decided female-producing tendency.

The seventh line of study intended to analyze the seasonal and clutch deliveries of the sex-controlled series is concerned with arrangements by which the sex-behavior of the birds from such series is tested. In these pro-

[11] The males, in both of these species, average 10–15 grams heavier than the females; the *risoria* birds are slightly larger on the average (5–10 grams) than *alba*.

[12] The weights given for individual birds represent the average of the monthly, or bi-monthly weights for the year.

cedures female is mated with female, and male with male. Such pairs—from a very few selected pairs of parents—are kept mated for a period of six months. The three and one half years that this study has been pursued has enabled us—using 30 to 50 birds—to test one and the same bird with seven others. Most of the birds used—for lack of success with the incessantly fighting males—have been females, and most of the seven successive tests with each bird have been made with its sisters of the same series. The members of the pairs are kept apart except when under observation; when put together—as they are twice daily—the records are taken of those females of the pair which behave as males in copulation with their mates. Three facts are definitely established by the data obtained: (1) The females of the *orientalis* × *alba* cross (they are dark in color) are more male-like in their sex behavior than the females of the reciprocal cross (these are white in color). (2) Females hatched from eggs laid earlier in the season are more masculine in their sex behavior than are their own full sisters hatched later in the season. *And, several grades of females can be thus seriated according to season of hatching.* (3) The female hatched from the first egg of the clutch is more masculine than her sister hatched from the second of the clutch in a great majority of the cases. And in nearly all these latter matings the more masculine bird is so decidedly so that she takes the part of the male a full 100 per cent. of the time in copulating with her very feminine clutch-mate sister.

A fuller account of this situation was given, with the assistance of charts too large to exhibit or describe here, before the Society of Zoologists in 1913.[13] The nature of this behavior has been adequately recorded by means of moving-picture films. Such records were also made showing the reversal of the known sex-behavior of such pairs by means of appropriate injections of ovarian and testicular extracts. Those films were demonstrated in this hall—or in one near-by—in connection with an ad-

[13] Abstract in *Science*, March 20, 1914.

dress before the local chapter of Sigma Xi some 20 months ago.

The injection of the extracts of gonads, performed now on the third series of birds, has resulted—quite against our wish—in the death of a number of birds. In the main the deaths from ovarian injections were of the more masculine birds; while the deaths from testicular injections have been among the more, or most, feminine birds. The numbers concerned at present are not large, and a further definite study of the matter will be made before final conclusions are drawn. But the limited data now at hand indicate that the eighth correlation listed on Chart 1 is as it is exhibited there.

A ninth, and very accurate and convincing kind of information concerning the germs involved in these sex-series has been obtained by means of the bomb calorimeter. The heat of combustion of some 200 egg-yolks has been determined. One such series of determinations for 1914, in which all available eggs were burned, is shown on Chart 4. It will there be seen that the first clutch of the season bore a higher caloric value than the second, but is otherwise the smallest of the year. Beginning with the second clutch laid in June, the succeeding clutches to December 1 bear higher and higher heat values. In all clutches too, except the very first, the second eggs show a higher storage of heat units than do the first of the clutch. Here we find the conclusions reached from studies on the weights of yolk, and on yolk analyses, fully confirmed by a study of the burning value of the materials stored. And confirmed by a method in which the error involved in the determination is wholly negligible. The most accurate method, for the study of the storage values of male- and female-producing ova, gives too the results most consistent with the breeding data.

The tenth and last of these correlations deals with embryological or morphological data. It was found that some females dead at relatively advanced ages showed persistent right ovaries. The right ovary in pigeons

normally begins degeneration at or before hatching and is wholly absent from the week-old squab. It soon became evident that the persistent ovaries were found practically exclusively in birds hatched from eggs of overworked series. Further study has shown in addition that they arise almost wholly from the eggs of autumn, and predominantly then from the second egg of the clutch—that is from eggs otherwise known to have the greatest or strongest female-producing tendency. These ovaries have sometimes weighed nearly a third as much as the adult left ovary with which they were associated, and have been found in such birds dead at all periods from a few days to fifteen months. We here attempt no adequate description of this situation, but one can not have observed the frequency of the persistence of this ovary in the birds hatched from the eggs otherwise known to be the most feminine from these overworked series without conviction that the same pressure which carries the eggs of spring from male-producing to female-producing levels, also carries the earlier female-producing level, to another yet more feminine.

In conclusion, the studies that have thus far been made on sex, and on the experimental control of sex, in pigeons go very far, we believe, toward an adequate demonstration that germs prospectively of one sex have been forced to produce an adult of the opposite sex—that germs *normally* female-producing have, under experiment, been made to develop into males; and that germs which were prospectively male-producing have been made to form female adults. That neither selective fertilization, differential maturation nor a selective elimination of ova in the ovary can account for the observed results. Further, and perhaps of more importance, these studies throw much new light on the nature of the difference between the germs of the two sexes. This difference seems to rest on modifiable metabolic levels of the germs; males arise from germs at the higher levels, females from the lower; and such basic sex differences are quantitative, rather than qualitative in kind.

THE CALCULATION OF LINKAGE INTENSITIES[1]

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Two methods of estimating the intensity of linkage are in use. One consists of crossing individuals heterozygous for two or more linked genes with homozygous recessives. This is the more direct method, because the gametic ratio—barring differential viability—is exhibited directly by the zygotic frequencies. The other method employs ordinary F_2 ratios derived from selfing F_1 or breeding together like F_1 individuals. Here the gametic ratio can only be inferred from the numerical relation of the zygotic classes. The results may be disturbed not only by differential viability, as in the first method, but also by selective fertilization, if that occurs, and may often be materially influenced by chance in random mating where the numbers are small. In fact, this method is so undesirable that it should not ordinarily be used where the other method is practicable. It is true, however, that the mechanical difficulties of crossing certain plants are so great and the number of seeds produced per flower so small that often the ordinary F_2 results are alone available. It is important, therefore, to have a means of calculating gametic ratios from F_2 zygotic numbers.

Since no direct formulæ for calculating gametic ratios from observed F_2 data have heretofore been available, the problem has been attacked in an indirect way. A series of F_2 zygotic ratios has first been calculated from a corresponding series of gametic ratios. Next the observed F_2 results have been compared with the calculated series, the closest fitting calculated ratio determined, and the corresponding gametic ratio taken as that responsible for the observed F_2 results.

[1] Paper No. 54, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

The method of determining the closeness of fit between calculated and observed numbers used by Bateson, Punnett and their co-workers was mere inspection. (See Bateson and Punnett, 1911.) The unreliability of this method was pointed out by Collins (1912) who made use of Yule's coefficient of association for the same purpose. The well-known formula for this coefficient is $(ad - bc)/(ad + bc)$, where a, b, c, d are the frequencies of the phenotypic forms AB, Ab, aB, ab, respectively. From a table giving the coefficients of association for a series of gametic ratios, the best fitting gametic ratio is chosen by inspection or interpolation. This method is satisfactory except for the higher gametic ratios where slight differences in the coefficients of association correspond to wide differences in the gametic ratios. Since the same intensity of linkage gives somewhat higher coefficients of association for coupling than for repulsion, particularly for the lower linkage values where the association coefficient method is most reliable, two tables must be used.

Formulæ, by which gametic ratios can be approximated directly from F_2 data without the use of coefficients of association and without respect to whether coupling or repulsion is involved, would seem to merit trial. Such formulæ are presented later in this paper. Moreover, it is often desirable to reverse the calculation, that is, to determine zygotic frequencies from assumed gametic ratios. A single formula suggested for this purpose gives accurate results for both coupling and repulsion. This formula will be presented first because the others are developed from it.

Bateson and Punnett (1911) suggested two empirical formulæ for calculating zygotic frequencies from assumed gametic ratios, one for coupling and the other for repulsion. Neither one, of course, is applicable to both types of linkage, though both formulæ are true for independent inheritance. If A and a are allelomorphic genes and B and b are a similar allelomorphic pair—the capital letters

denoting dominance—and if $2n$ equal the sum of the gametic series,[2] then the gametic series and the phenotypic zygotic series, *AB, Ab, aB, ab,* for coupling and for repulsion are:

	Gametic Series			
	AB :	*Ab* :	*aB* :	*ab*
Coupling	$n-1$:	1:	1:	$n-1$
Repulsion	1:	$n-1$:	$n-1$:	1

	Zygotic Series			
	AB :	*Ab* :	*aB* :	*ab*
Coupling	$3n^2-(2n-1)$:	$2n-1$:	$2n-1$:	$n^2-(2n-1)$
Repulsion	$2n^2+1$:	n^2-1 :	n^2-1 :	1

That is, the formulæ of Bateson and Punnett are expressed in terms of the sum of the gametic series. But the same thing can also be expressed in terms of the several members of the gametic series. Thus, if $r:s$ is any gametic ratio, the usual form of gametic series is $r:s:s:r$ and the frequencies of the ten possible genotypic classes and of the corresponding four phenotypic classes are:

Genotypes	Phenotypes
$AB \cdot AB = r^2$	
$AB \cdot Ab = 2rs$	
$AB \cdot aB = 2rs$	$AB = 3r^2 + 4rs + 2s^2$
$AB \cdot ab = 2r^2$	
$Ab \cdot aB = 2s^2$	
$Ab \cdot Ab = s^2$	$Ab = 2rs + s^2$
$Ab \cdot ab = 2rs$	
$aB \cdot aB = s^2$	$aB = 2rs + s^2$
$aB \cdot ab = 2rs$	
$ab \cdot ab = r^2$	$ab = r^2$

The general formula for calculating a phenotypic zygotic series from a given gametic ratio is, therefore,

$$3r^2 + 2(s^2 + 2rs) : s^2 + 2rs : s^2 + 2rs : r^2 \qquad \text{(I)}$$

The sum of the zygotic series is $4r^2 + 8rs + 4s^2$ or $(2r + 2s)^2$, which, when expressed as

$$(r + s + s + r)(r + s + s + s + r),$$

[2] Bateson and Punnett considered n to be some power of 2, but this limitation need not apply here.

indicates how the formula is derived. Reference to the diagram will make this clear. Since r and s are any positive quantities, formula I is applicable to coupling

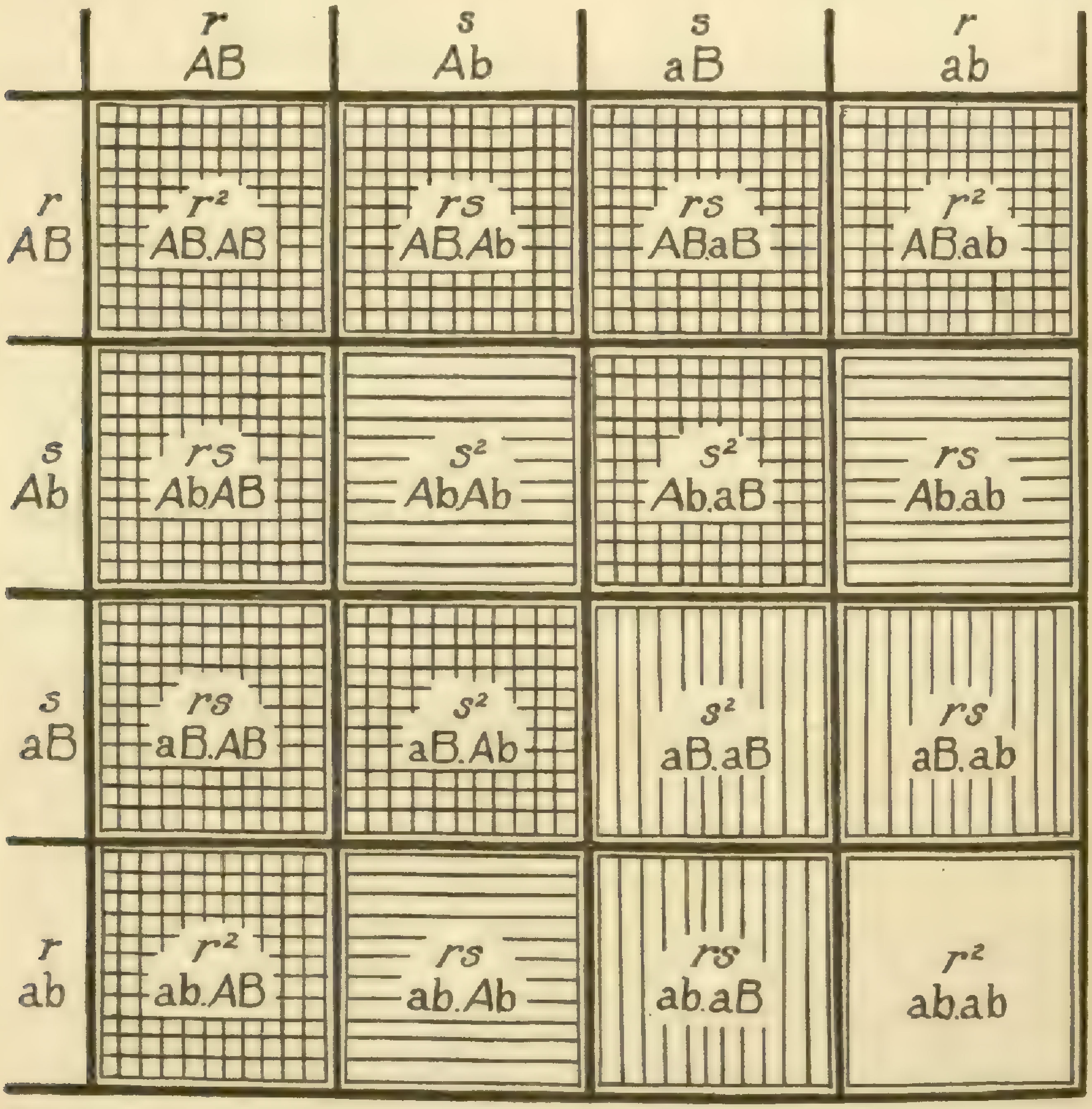

DIAGRAM SHOWING IN TERMS OF r AND s THE NUMERICAL RELATIONS OF THE F_2 ZYGOTIC CLASSES THAT RESULT FROM COMBINATIONS OF THE GAMETIC CLASSES *AB, Ab, aB, ab* OCCURRING IN THE RATIO SERIES $r:s:s:r$. The dominant genes *A* and *B* are indicated by horizontal and vertical lines respectively, while their allelomorphs *a* and *b* are indicated by the absence of such lines. (See formula I.)

$(r > s)$, repulsion $(r < s)$ and to independent inheritance $(r = s)$. It, of course, gives the same result as the empirical formulæ of Bateson and Punnett, but is more convenient in that one formula takes the place of the two. It is easy to use since the fourth term of the zygotic series is the square of r, the second and third terms each the square of s plus twice the product of r and

s, and the first term the sum of the second and third plus three times the fourth.

An approximation of gametic ratios can be obtained from observed zygotic ratios by simple formulæ derived from formula I. If the actual values of $s^2 + 2rs$ could be assumed to be identical in all cases, it would follow from formula I that $4r^2 = AB + ab - (Ab + aB)$ and $r = \sqrt{(AB + ab - Ab - aB)/4}$. Similarly, $4(s^2 + 2rs) = AB + Ab + aB - 3r^2$ and $s = \sqrt{(AB + Ab + aB + r^2)/4} - r = \sqrt{(AB + Ab + aB + ab)/4} - r$. If E is the sum of the extreme terms and M the sum of the middle terms of the observed zygotic series, the formulæ for approximating gametic ratios are, then,[3]

$$\begin{aligned} r &= .5\sqrt{E - M} \\ s &= .5\sqrt{E + M} - r \end{aligned} \qquad \text{(II)}$$

If it is desired to compare the observed F_2 frequencies with a calculated series of frequencies, the procedure, obviously, is to calculate the gametic ratio by formulæ II —or by means of the coefficient of association—and then to calculate the zygotic series by formula I—or by one of the two formulæ of Bateson and Punnett. This procedure is not always necessary, however, for a theoretical zygotic series can usually be readily computed directly from the observed frequencies. If AB, Ab, aB, ab is the series to be calculated from the observed frequencies, it follows from formulæ I and II that

$$\begin{aligned} Ab = aB &= M/2 \\ ab &= (E - M)/4 \\ AB &= M + 3ab \end{aligned} \qquad \text{(III)}$$

Since a zygotic series calculated in this way necessarily meets the conditions imposed by formula I, the gametic ratio can be approximated from it more readily than from the observed frequencies. Since by formulæ I and II

$ab = r^2$ and $s = .5\sqrt{E + M} - r$,

$$\begin{aligned} r &= \sqrt{ab} \\ s &= .5\sqrt{E + M} - \sqrt{ab} \end{aligned} \qquad \text{(IV)}$$

[3] Since r and s are necessarily positive, negative roots are disregarded.

Formulæ IV are not to be used in connection with observed F_2 frequencies except when the latter approximate closely the form demanded by formula I, that is, when the first term of the observed frequencies equals approximately the sum of the second and third terms plus three times the fourth term.

In cases of repulsion, where the fourth term of the zygotic series is always relatively small and, therefore, where the first term should be only slightly greater than the sum of the second and third terms, it may happen that the sum of the first and fourth terms, E, is actually less than the sum of the second and third terms, M. In such cases, formulæ II (and consequently formulæ III and IV also) can not be employed, for, if E is less than M the quantity under the radical $(E - M)$ is negative and has no real root. In such cases, the gametic ratio must be calculated by means of the coefficient of association.

The method here suggested for calculating gametic ratios from observed frequencies never gives quite the same results as that obtained by the association-coefficient method except when the observed series approaches closely the form demanded by formula I. Naturally, then, the more widely the observed frequencies depart from this form the greater the difference between the results given by the two methods. Since the coefficient of association gives reliable results if the tables to be used with it are based upon sufficiently small differences in the gametic ratios employed in its preparation, it follows that the methods proposed in this paper give only approximate results. It is also true, therefore, that the nearer the observed frequencies approach the form of formula I, the closer the approximation obtained by formulæ II (or III and IV).

The two methods have been applied to numerous cases taken from published accounts of linkage studies and the goodness of fit tested by the method suggested by Harris (1912). The differences, $o - c$, between the observed frequencies, o, and the calculated frequencies, c, of the sev-

eral classes are determined and $S[(o-c)^2/c]=x^2$ calculated, S indicating summation.

With n, the number of classes, here equaling four, and x^2, the probability, P, that departures from the calculated series as great as those observed might occur through the errors of random sampling, is obtained by reference to Elderton's (1901) table (see also Pearson, 1914). Wherever appreciably different gametic ratios have been obtained by the two methods, P has been found to be greater for the association-coefficient method than for the method based on formulæ II. The former method has, therefore, given the closer fit. Since, in most of the cases to which the test has been applied, x^2 is less than one and since such values are not listed in Elderton's table, x^2 has been used directly for the comparison of the two methods. Where n is constant, the larger x^2 the less the probability.

While this test for goodness of fit has shown the association-coefficient method to be the better of the two, the fact that in most cases x^2 was less than one for both methods indicates that the approximate method suggested here ordinarily gives results such that the departures of observed from calculated frequencies might well be due to errors of random sampling. The method has been found convenient and usually sufficiently accurate where only an approximate determination of the gametic ratio is desired. Where the observed frequencies depart widely from the form given by formula I, this method should not be used. It should be noted, however, that in such cases no calculated series fits the observed results well. This limitation to the use of the new method does not lessen materially the convenience of using it where it is applicable. By a mere inspection of the observed frequencies, it can usually be told whether they conform fairly closely to formula I, that is, whether the first term is approximately equal to the sum of the second and third plus three times the fourth.

A few examples will illustrate the use of the approximate method of calculating gametic ratios from observed

data and afford a means of comparing it with the association-coefficient method.

Harris (1912) has quoted an example of coupling in sweet peas from the studies of Bateson, Saunders, and Punnett[4] and calculated P where the gametic ratios are taken as 7:1 and 15:1, the only ratios considered in the original paper. The phenotypic classes are based on shape of pollen and color of flowers and the observed frequencies are purple long 493, purple round 25, red long 25, red round 138, total 681. As determined by Harris, on the basis of a 7:1 gametic ratio, $P = .0053$ or $x^2 = 12.7699$. On the 15:1 basis, $P = .3086$ or $x^2 = 3.6375$. The chances against the 7:1 ratio are, therefore, 199 to 1 and against the 15:1 ratio about 2 to 1. For this same material, Collins (1912), using the association-coefficient method—Coef. Assoc. $= .982 \pm .004$—naturally suggested a 12:1 gametic ratio—Coef. Assoc. also $= .982$—and pointed out the fact that the deviation from the 7:1 ratio is 9 times and from the 15:1 ratio about twice the probable error. By formulæ III, the calculated series becomes $485.75 + 25.0 + 25.0 + 145.25 = 681$. By formulæ IV, $r = 12.052$ and $s = .996$ or a gametic ratio of 12.1:1. The 12:1 ratio obtained by the association-coefficient method gives a zygotic series of $485.5 + 25.2 + 25.2 + 145.1 = 681$. Both methods, then, give gametic ratios approximately the same and practically identical zygotic series, namely, $485 + 25 + 25 + 145$. On the basis of this series, $x^2 = .4387$ and P is so large that it is useless to determine it. In short, both methods give gametic ratios that fit the observed data extremely well.

The next example of coupling presents a very different condition. It has been quoted by Bridges (1914) from Punnett's (1913) summary of reduplication series in sweet peas. The phenotypic classes are based upon sterility of anthers and form of flowers and the observed frequencies are fertile normal 165, fertile cretin 58, sterile normal 58, sterile cretin 78, total 359. It can be seen at a glance that these frequencies are far from

[4] Rept. Evol. Com., 4: 11.

what formula I demands—$58 + 58 + 3(78) = 350$, over twice 165—and that therefore the approximate method can not be depended upon in calculating the gametic ratio. It is interesting to note, however, just how unreliable it is in comparison with the association-coefficient method. By formulæ III and IV, the calculated zygotic series becomes $211 + 58 + 58 + 32 = 359$, $r = 5.6$, $s = 3.8$, and the gametic ratio is approximately 1.5 : 1. Bridges referred the case to a 2 : 1 ratio (Coef. Assoc. = .558), though the coefficient of association is .588 which is equivalent to a gametic ratio of 2.1 : 1 (Coef. Assoc. = .586). Punnett compared the observed frequencies with a series derived from an assumed 3 : 1 ratio. The zygotic series calculated from these ratios are, for the 2 : 1 ratio, $219 + 50 + 50 + 40 = 359$; for the 2.1 : 1 ratio, $220 + 49 + 49 + 41 = 359$; and for the 3 : 1 ratio, $230 + 39 + 39 + 51 = 359$. If now the criterion of goodness of fit be applied to the four calculated series the values of x^2 are, for the 1.5 : 1 ratio 76.1, for the 2 : 1 ratio 52.0, for the 2.1 : 1 ratio 51.4, and for the 3 : 1 ratio 51.3. Values of x^2 above 30 are not listed in Elderton's table, but where $x^2 = 30$ and $n = 4$, $P = .000,001$, which means that there is only one chance in a hundred thousand of deviations so great as the observed ones being due to the errors of random sampling. Where neither of the two methods of calculating the zygotic series gives a better fit than in this case, it is immaterial which fit is the worse.

As an example of repulsion, the same characters, in sweet peas may be used. The observed frequencies (Bateson and Punnett, 1911) are $336 + 150 + 143 + 11 = 640$. Bateson and Punnett assumed that the gametic ratio concerned was 1 : 3. The coefficient of association is −.706, which is equivalent to a gametic ratio of 1 : 2.74. By formulæ II–I or III–IV, a ratio of 1 : 2.45 is indicated. The values of x^2 are for the 1 : 2.45 ratio .649, for the 1 : 2.74 ratio .302, and for the 1 : 3 ratio .536. Here again the association-coefficient method gives the better fit, but the probability is great that the deviations of the observed from the calculated frequencies, even in case of

the approximate method, might be due to errors of random sampling.

As an illustration of the fact that the approximate method can not be used in some cases of repulsion, even when the observed frequencies fit fairly well the series calculated by the association-coefficient method, an example of linkage between dark axils and fertile anthers in sweet peas quoted from Punnett by Bridges (1914) may be taken. The observed frequencies are $1335 + 643 + 714 + 2 = 2694$. The value of r can not be determined by formulæ II nor by III and IV, because $1335 + 2 - (643 + 714)$ is a negative quantity (-20) and has no real root. The coefficient of association is $-.988$, which is equivalent to a gametic ratio of 1:17, though Bridges assumed a ratio of 1:20. On the basis of this 1:20 ratio, $x^2 = 5.68$ and $P = .1309$. On the basis of the 1:17 ratio, $x^2 = 4.04$ and $P = .2615$, or odds of about 3 to 1 against the occurrence of deviations as great as those observed.

It may be said, then, that the formulæ suggested here afford a convenient method of approximating gametic ratios from zygotic series, when the observed frequencies are in fair accord with a series based on formula I—or the formulæ of Bateson and Punnett. When the observed frequencies are far from this type no method gives a close fit between observed and calculated results.

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THE MECHANISM OF CROSSING-OVER. IV

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THE "map" of the first chromosome, based on these experiments, is shown below:

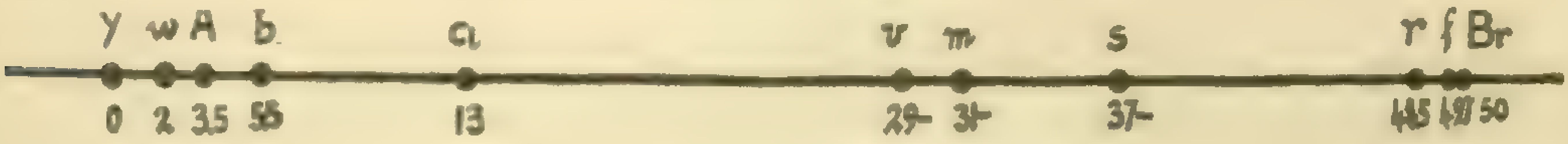


FIG. 10. Map of chromosome I.

The figures represent the distances of the factors from yellow, the first one in the line, and are calculated merely by adding together the intermediate distances. This map gives almost exactly the same proportionate distances between the different loci as does that obtained by combining the results of linkage experiments performed by other workers, in which usually the inheritance of only two or three factors was followed at one time. Each set of ratios, therefore, confirms the accuracy of the other. The absolute distances in the present map are, however, somewhat shorter, being 6/7 the length of those in the composite map. This was caused mainly by the comparatively large number of non-cross-overs produced by a few females; in the rest, the crossing-over frequencies were about normal. It may, therefore, be concluded that chromosomes which differ in regard to eleven pairs of factors behave in the same way, so far as crossing-over is concerned, as those which are alike except for two factors. This is contrary to a suggestion made by Punnett. Moreover, the fact that chromosomes differing in so many factors behave normally is here especially noteworthy, because 11 of the 12 recessive factors were in the same chromosome.

The results of the experiments with the second chromosome may now be tabulated. 462 offspring of females

heterozygous for the ten mutant factors used in this group have been recorded. The table only gives the result with respect to nine characters, however, as arc wing was not followed in all of the experiments. (The data given later as to its position have, accordingly, not been calculated from quite as large a count of flies as have the data for the other factors.)

CLASSIFICATION OF FACTOR COMBINATIONS TRANSMITTED BY FEMALES HAVING THE COMPOSITION: $\frac{S_{tr}\ b_l\ p_u\ v_g\ a_r\ s_p}{d_a j \qquad c_v\ \ b_a}$

Between	Streak		Not Streak		Total
Non-cross-overs					
	$Sbpvas$	68	$djcb$	82	150
Single Cross-overs					
S_{tr} and d_a	$Sd\,j\;cb_a$	11	$b\,pv\,s$	15	26
d_a and b_l	$S\;j\;cb_a$	24	$db\,pv\,s$	19	43
b_l and j	$S\,bj\;cb_a$	1?	$d\;pv\,s$	0	1?
j and p_u	$S\,b\;cb_a$	3	$d\,jpv\,s$	6	9
p_u and v_g	$S\,b\,p\;cb_a$	14	$d\,j\,v\,s$	20	34
v_g and c_v	$S\,b\,pv\,cb_a$	10	$d\,j\;s$	11	21
c_v and s_p	$S\,b\,pv\;b_a$	51	$d\,j\;cs$	50	101
s_p and b_a	$S\,b\,pv\,sb_a$	0	$d\,j\;c$	0	0
Double Cross-overs					
S_{tr} and d_a; p_u and v_g	$Sdj\;v\,s$	2	$b\,p\,cb_a$	1	3
S_{tr} and d_a; v_g and c_v	$Sdj\;s$	1	$b\,pvcb_a$	0	1
S_{tr} and d_a; c_v and s_p	$Sdj\;cs$	5	$b\,pv\,b_a$	8	13
d_a and b_l; j and p_u	$S\,j\,pv\,s$	1	$db\;cb_a$	0	1
d_a and b_l; p_u and v_g	$S\,j\;v\,s$	5	$db\,p\,cb_a$	5	10
d_a and b_l; v_g and c_v	$S\,j\;s$	5	$db\,pvcb_a$	1	6
d_a and b_l; c_v and s_p	$S\,j\;cs$	5	$db\,pv\,b_a$	8	13
j and p_u; p_u and v_g	$S\;b\;v\,s$	1	$d\,jp\,cb_a$	1	2
j and p_u; v_g and c_v	$S\;b\;s$	0	$d\,jpvcb_a$	1	1
j and p_u; c_v and s_p	$S\;b\;cs$	7	$d\,jpv\,b_a$	1	8
p_u and v_g; v_g and c_v	$S\;bp\;s$	0	$d\,j\;vcb_a$	2	2
p_u and v_g; c_v and s_p	$S\;bp\,cs$	3	$d\,j\;v\,b_a$	3	6
v_g and c_v; c_v and s_p	$S\;bpvcs$	2	$d\,j\;b_a$	1	3
Triple Cross-overs					
S_{tr} and d_a; d_a and b_l; c_v and s_p	$Sd\,bpvb_a$	1	....		..
S_{tr} and d_a; j and p_u; c_v and s_p	....		$b\;cs$	1	..
d_a and b_l; j and p_u; p_u and v_g	$S\,j\,pcb_a$	1	....		..
d_a and b_l; j and p_u; c_v and s_p	....		$db\;cs$	1	..
d_a and b_l; p_u and v_g; c_v and s_p	....		$db\,p\,cs$	1	..
j and p_u; p_u and v_g; c_v and s_p	....		$d\,jp\,cs$	1	..

Total Single, Double, and Triple Crossing-over

Between	Observed Number	Per Cent. of Crossing-over
S_{tr} and d_a	45	9.7
d_a and b_l	77	16.7
b_l and j	1?	0.2?
j and p_u	25	5.4
p_u and v_g	59	12.8
v_g and c_r	34	7.1
c_r and s_p	150	32.5
s_p and b_a	0	0.0

In the case of this chromosome, too, the law of linear linkage is graphically illustrated by the characteristic "sectional" mode of interchange between the groups. The non-cross-overs here constitute only 32.5 per cent. of the population, whereas the single cross-overs make up 51.1 per cent., the double cross-overs 15.2 per cent., and the triple cross-overs 1.3 per cent. In making a map of this chromosome, the chances of error are greater than in the preceding case, since not so many flies have been obtained. Nevertheless, the values correspond very closely with estimates of the results obtained in other work, although figures exactly representing the sum total of other work are not just now available for comparison.

FIG. 11. Map of chromosome II.

Let us now construct a curve showing the frequency with which, in the experiment with the first chromosome, points various distances apart showed coincidence of crossing-over. Suppose that in this curve the horizontal line represents the distance apart of the two coincident cross-overs, and the vertical line the per cent. of cases in which double crossings-over at such distances occur. For example, if it were known that double crossing-over for a distance anywhere between 15 and 16 units occurred in .2 per cent. of all cases the height of the curve above the figures 15 and 16 would be made .2 vertical units. Now, each case of double crossing-over that actually happens

among the 712 flies obtained for group I must represent $\frac{1}{712}$, or .14 per cent., of all the cases. If, then, a crossing-over is found to occur somewhere between c_l and v, and one occurs coincidentally between s and r, the two points of crossing-over may have been as far apart as c_l and r (36), or as close together as v and s (8), or at any intermediate distance. Therefore we have no right to make this case stand, in the curve, for a coincidence that happened at a particular distance (say 10–11) and to raise the ordinates for this particular distance by .14 units. Each distance between 8 and 36 is consequently given partial credit in our curve for the occurrence of this coincidence, and so each of the 28 ordinates between 8 and 36 is raised to an average height of $\frac{.14}{28} = .005$ approximately. All the other cases are treated in a similar way, and thus the curve shown by the heavy line in Fig. 12 is obtained.

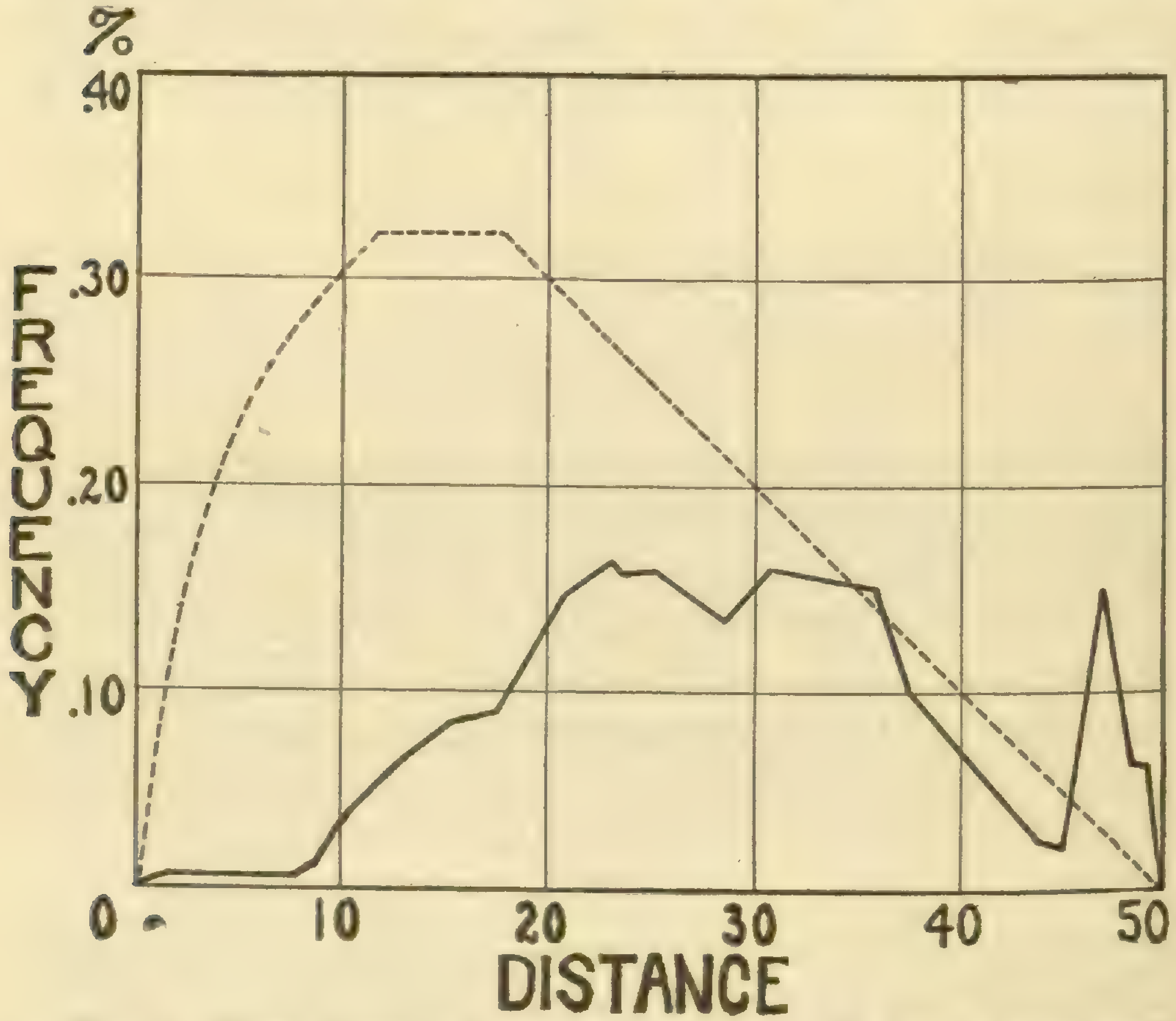
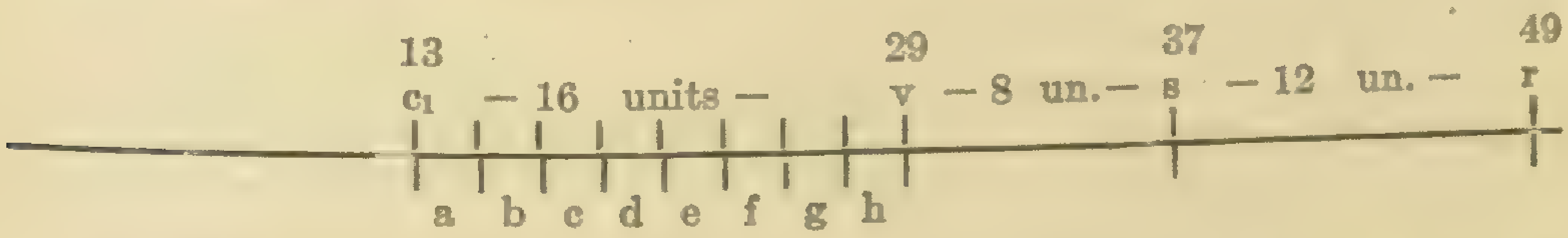


FIG. 12. Curve showing the observed frequency of double crossing-over in chromosome I, for points various distances apart. The dotted line shows the frequency expected on pure chance.

But although these ordinates are, on the *average*, raised by this amount, each one is not raised equally, for there is less chance that double cross-overs should have the most extreme possible values than medium values. The total addition of .14 units to the curve should hence be distributed among the different possible ordinates according to the relative probabilities that the two points of crossing-over should have been the distance apart represented by these respective ordinates. These various probabilities for the different ordinates, in the case of any specific double cross-over, may be represented in the form of a curve, and the main curve of double cross-over frequency shown in figure 12 is thus really a composite in which these individual curves for each double cross-over have been added together. We may now consider the way in which the individual curves of probability are calculated.

Let us take the case of the double cross-over that occurred between c_1 and v and coincidently between s and r. We have already calculated that the distance between the two points of crossing-over must be somewhere between 8 units and 36 units (see second paragraph above). The curve for this individual double cross-over will therefore start at 8 on the abscissa and continue to 36. What height shall it have along the ordinates between these points? Let the region c_1 — v be divided into 8 equal parts —abcdefgh—of two units each, as shown below.



It will be seen that a double cross-over of 8 to 10 units length (*i. e.*, having 8 to 10 units between its two points of crossing-over) which passes between the factors c_1 and v, must go between them in the region h, if its other point of crossing-over is to be between s and r. However, any double cross-over of 10 to 12 units length which passes through either g or h will also pass between s and r, and so there is twice as much chance for double cross-overs of

this length to occur as for those 8 to 10 units long. Similarly, those 12–14 units long may be three times as numerous, for they may pass through f, g, or h, and so with each increment of length, up to 20, there will be an equal additional amount of chance for a double cross-over of that length (passing through the required sections, $c_1 - v$ and $s - r$) to occur. Thus our curve of probability rises in regular steps from 8 to 20; if we could have divided the distance $c_1 - v$ into an infinite number of parts, instead of into 8, these steps would each be infinitely small, and so we should have a straight line rising from 8 to 20.

Beyond this point the rise in probability ceases; a double cross-over between 22 and 24 units long has no more chance of happening than one of 20–22 units. Reference to the figure will show that a double cross-over of 20–22 units passing through any of the regions from c through h will separate s from r and thus fulfill the requirements, but a double cross-over 22–24 units long, while it has the additional alternative of passing through b, can not pass through h without its second point of crossing-over falling to the right of section $s - r$. Similarly, one 24–26 long may not pass through g or h, though it may pass through any region from a to f; double crossovers of all these lengths therefore have the same chance of occurring, and our curve along the corresponding ordinates would hence be a horizontal line.

Double cross-overs longer than this would have less and less chance of occurring; one 26–28 long could only pass through regions $a - e$, one 28–30 only through $a - d$, and so the curve falls again in a straight line to the zero level at 36.

The same rules can be shown to apply to all cases: the curve starts at a place on the abscissa representing the distance apart of the innermost factors involved (in the above case this distance was $v - s$, $= 8$); it rises in a straight line for a distance equal to the length of the smaller section involved (above, this was the distance $s - r$, $= 12$, so that the line rose to point $8 + 12$, $= 20$);

it then proceeds horizontally until a distance from the starting point of the curve equal to the length of the longer section has been passed (above, this was the section $c_1 - v$, $= 16$; thus the line proceeded on a level to point $8 + 16 = 24$[6]); then it falls in a straight line to a point on the abscissa representing the distance between the outermost factors involved (above, the distance is $c_1 - r$, $= 36$). The height to which the curve rose is determined by the fact that its area (the sum of all the ordinates) must have a value representing the per cent. of total cases in which such a double cross-over occurred (above, each double cross-over must have a curve with an area $= .14$, since each fly was .14 per cent. of the total count).

It will be noted that for each individual curve the probability is calculated on a basis of pure chance, no account being taken of possible interference, which, if present, would tend to make the longer distances more likely than the shorter, and so to raise the right end of the curve at the expense of the left. In other words, each *individual* curve represents the frequency with which double cross-overs of different lengths would happen *within* the particular regions dealt with (in our case above, regions $c_1 - v$ and $s - r$), if there were no interference and they had a purely chance distribution, within these regions. The *composite* curve thus errs rather by showing too little effect of interference than too much. All interference which it does show—that is, all deviation between it and a curve representing an entirely random distribution of double cross-overs—must then be due solely to the way in which the double cross-overs were found to be distributed *among* the various regions, as no assumption of interference was made in calculating out the curve for each double cross-over.

The curve representing the proportion of double cross-overs of different lengths which would have been found on an entirely random distribution (no interference) is

[6] The discrepancy between this figure (24) and that (26) found by the method of trial used above would disappear if the region $c_1 - v$ had been divided infinitely instead of only into eight parts.

shown by the dotted line. To make comparison with the other curve legitimate, it had to be constructed by the same method,—namely, by making a composite of individual curves, each of which represented the probabilities for a certain type of double cross-over—only, instead of using the observed numbers of double cross-overs of the different types, in constructing it, it was necessary to use the numbers of double cross-overs of the different types that would have been observed if there had been no interference. (This curve hence represents the results of a chance distribution both among and within the various regions.) In the case of each type of double cross-over, the way to find the per cent. of individuals showing it that would be produced if there were no interference, is to multiply the total per cent. of crossing-over in the first region by the per cent. in the second region, as explained in section 4a. (Thus, the per cent. of double cross-overs passing between A and B and between C and D equals per cent. of cross-overs between A and B times per cent. of cross-overs between C and D.) This per cent., then multiplied by the total number of individuals counted, gives the number of such double cross-overs theoretically to be expected in the absence of interference. When such calculations for each different possible kind of double crossover have been made, and the individual curve for each then made, the latter may be combined to form a composite curve like the curve shown by the dotted line.

The end desired is of course to compare the dotted and the heavy-lined curves and see what proportion of the double cross-overs various distances apart, that were expected on pure chance, actually occurred. Therefore a new curve (Fig. 13) may be made, representing this *relative coincidence, i. e.*, the per cent. which each frequency on the observed curve formed of each frequency on the expected curve (see sect. IVa). This curve consequently shows the rise or fall of the index with which we are already familiar, and which we have called simply "coincidence."

Owing to the fact that not very large figures have so far

been obtained, we must be cautious about accepting the exact values shown in the curve of coincidence; this applies not so much to the main portion of the curve as to the right-hand end (shown in dotted lines), for in the case of very long double cross-overs, very few kinds are even theoretically possible, compared to the number of different

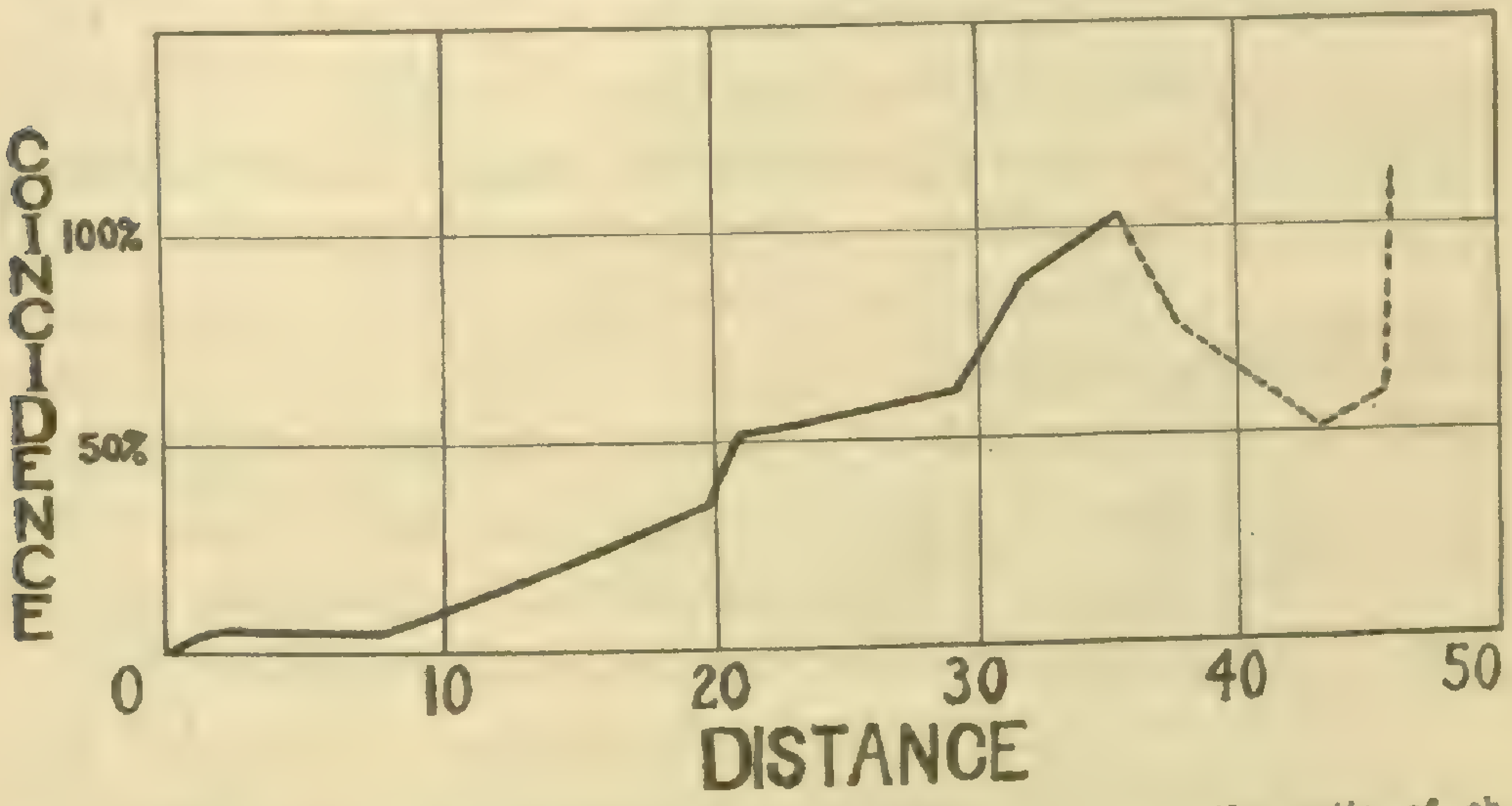


FIG. 13. Curve of coincidence for chromosome I, *i. e.*, the ratio of observed double crossing-over for points in chromosome I various distances apart to double crossing-over expected on a chance basis.

positions in which short double cross-overs of a given length may be found. Accordingly, the marked fall, followed by great rise at the very end of the first curve has no true significance.

Certain points may be seen to stand out plainly, however. It is clearly evident that interference is great for short distances—*i. e.*, that relative coincidence is low; as distance increases the coincidence rises, at first, quickly, but beyond a certain point the rise ceases.

There is no indication of a usual length of loop of less than half the length of the chromosome, as cytological observations on strepsinema stages would suggest, and as would therefore be expected on the view that crossing-over occurs at that stage. The fall seen near the right hand end is entirely unreliable, as has been explained. But, even if taken at its face value, the drop at this point can have no significance for the question at issue, for a fall due to the loop would have to be as long as the whole pre-

vious rise. In addition, the curve should, on this explanation, rise high above the 100 per cent. level at its modal point, whereas it is evident that, so far as the significant figures go, it does not rise much above 100 per cent. at any point. It would be premature, however, to generalize further on these results.

The curve for group II will not be presented until greater numbers of flies have been recorded. It may be stated, however, that this curve too shows the phenomenon of interference, although, since the factors are not so close together, the crossing-over for rather small distances cannot so well be followed.

The great variability possible in the distance between two points of crossing-over is shown not only in the above curves, but may be graphically illustrated from a single case. This fly was the triple cross-over in the first chromosome, which has already been mentioned. Its mother was one of the tested females of the count, whose composition proved to have been $\frac{\text{ywAbvmsrf}}{B_r}$, and it itself was a male with the factors yrB_r. Crossing-over, therefore, must have taken place between y and w, s and r, and r and f. The minimum possible distance between the first two points of crossing-over is 42, the maximum distance between the second two is 14. The latter is the smallest distance ever observed between two points of crossing-over. It may here be mentioned that it will be of great interest, when more extensive figures are obtained, to see whether in the second chromosome the same coincidence holds between crossings-over on opposite sides of the middle point as between crossings-over an equal distance apart, but on the same side. The bend of the chromosomes in the middle, or some other structural difference here due to the attachment of the spindle fiber at this point, might cause the results to be different in the above two cases.

Incidentally, the results demonstrate another point, lying in a somewhat different field of genetics. By following the method of keeping stocks constantly in heter-

ozygous condition, twenty-two factors have been continually outcrossed, in each successive generation, to their allelomorphs. Yet after about seventy-five generations of outcrossing, these characters do not show the slightest contamination. The experiment therefore forms an extensive test and verification of the "purity of Mendelian segregation." Castle has, however, raised the point that in determining whether characters change, we should not be content with casual inspection. One of the characters in the above experiment—dachs legs—lends itself readily to quantitative work, since one of its main features is a shortening of the tarsus and metatarsus. Measurements of the legs of about a dozen of these dachs flies, derived from the stock which had been subjected to continual outcrossing, were therefore made, as well as measurements of the legs of some dachs flies derived from a stock which had been kept pure; the values for normal flies were determined also. At the same time the thorax length of the flies was observed, in order that any difference in leg length due merely to variation in the size of the whole animal might be allowed for. The results for each individual are shown in the following table. Measurements are given in eyepiece micrometer divisions, each of which represented .026 mm.

In order to discover whether the character had become more variable as a result of outcrossing, the standard deviation of the ratios of foot to thorax, in the two stocks of dachs, was calculated from the above data. In the outcrossed stock the standard deviation was found to be .036, and in the original stock .035; that is, so far as these results can show, the variability of dachs after outcrossing has remained just the same. However this may be, the fact remains that the character, after being subjected to such long-continued outcrossing, had not approached one whit nearer to the type of its allelomorph. The slight difference in the other direction observed between it and the original mutant stock is of no significance, since just about as great differences in thorax length occurred between the

two stocks, but in opposite directions in the two sexes. The judgment based upon measurements accordingly confirms the judgments based upon inspection.

FEMALES

Dachs from Outcrossed Stock		*Dachs from Uncrossed Stock*		*Wild Flies*	
Length of Thorax	Length of Foot (Tarsus Plus Metatarsus)	Thorax	Foot	Thorax	Foot
32	19	32	20	41	31.5
35	20	33	21	42	31
35	20	34	19	42	31
36	19.5	35	20	42	31
36	20	35	20.5	43	32
36	20.5	35	21	43	33
				44	34
Averages:					
35	19.8	34	20.25	42.4	31.9
Ratio of foot to thorax length: .567		.596		.752	

MALES

Dachs, Outcrossed		*Dachs, Uncrossed*		*Wild*	
26	18	28.5	17.5		
28	19	29	19.5	26	24
29	20	29	20.5	29	26
30	21	30	19	29	26
31	17.5	32	22	32	28
31	19	32	23.5	33	29
32	20	33.5	23	37	32
Averages:					
29.6	19.2	30.6	20.7	31	27.5
Ratio of foot to thorax: .650		.677		.887	

Summary

1. Recent results complete the parallelism between factor groups and chromosomes in *Drosophila*. This strengthens the evidence that separation of linked factors is due to an *interchange between chromosomes*.

2. The chief gaps in the information regarding the total frequency of interchange in the different groups have been filled, and it is found that the usual total frequencies of separation correspond to the lengths of the chromosomes. This constitutes specific evidence that *crossing-over is the method of interchange* between the chromosomes, and that

the frequency of crossing-over between factors is determined by their distance apart in the chromosome. It supplements the other evidence for these conclusions that had previously been found by Sturtevant in the linear manner of linkage of the factors.

3. It seems uncertain whether crossing-over occurs in the strepsinema stage, as concluded by Janssens, or earlier in synapsis. The cytological evidence at present at hand would seem insufficient to settle this point. Possible tests for various alternative mechanisms of crossing-over are proposed.

4. In order to study the nature of crossing-over by means of "interference," stocks were made up that differed in regard to many factors. Females heterozygous for 22 pairs of factors were thus obtained, and a special method was devised for testing their output. Other special methods for obtaining multiple stocks, and for eliminating discrepancies due to differential viability, have also been presented.

5. The results have been arranged in the form of a curve showing the amount of interference for various distances. The results thus far obtained confirm those obtained by less exact methods, and also give evidence that interference decreases gradually with distance from a point of crossing-over; this, taken together with certain evidence from non-disjunction, lends some probability to the view that crossing-over occurs at an early stage in synapsis.

6. A case of crossing-over in an embryonic cell of a male is reported.

7. Incidentally, the experiments have afforded an extensive test of Castle's assumption of contamination of factors by their allelomorphs. Outcrossing in each generation for 75 generations has failed to change any of the factors.

The author is deeply indebted to Professor Morgan, and wishes also to convey his appreciation of the active cooperation so often rendered him by E. R. Altenburg and

A. H. Sturtevant, who, moreover, on several occasions helped to tide the stocks over critical periods during which it was not possible for the author to carry on the work. Thanks are also due to C. B. Bridges, for supplying several multiple stocks as well as for the use of a number of mutants which he had already located but an account of which he has not yet published.

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SHORTER ARTICLES AND DISCUSSION

DISTRIBUTION OF THE CACTI WITH ESPECIAL REFERENCE TO THE RÔLE PLAYED BY THE ROOT RESPONSE TO SOIL TEMPERATURE AND SOIL MOISTURE

As is very well known, it is the common habit, when referring to the relation of a "plant" to its environment, to mean the subaerial portion only, leaving quite to one side the subterranean parts. That there is little logic in this will be readily acknowledged, although the possible causes are not far to seek. In the first place, for patent reasons, roots do not greatly excite our admiration or curiosity, and thus have received little attention in the field. Further, relatively little experimental work has been done on the roots of plants other than on seedlings and growing in solutions. And besides these conditions which refer immediately to the plant, there is a nearly related one which has to do with its environment, especially with the root environment. The soils and the soil condition of whatever sort are probably more difficult to study, and the results more difficult to express in a manner capable of ready application than the subaerial environment of the plant. However, it has not been its difficulty alone that has been the deterrent in the study of the environment of roots since certain features, for instance the soil temperature, can be easily learned by appropriate apparatus. Could we have a comprehensive series of data touching this feature alone, to mention no other, we should be in possession of a very useful engine for use in comparative studies on causes underlying the distribution of plants, and, further, through it the study of the root-systems of plants, and of their biological value, would be greatly stimulated.

While it is here recognized that the presence of a plant in its environment is an expression of the response of the whole plant to the entire environment, it is necessary, for the purpose in hand, to ignore the responses of the shoots, and to focus our attention for the time on the root relation alone. It can be noted, however, as is very well known, that the activities of the latter may be reflected in those of the former. Such a condition, having inter-

esting possibilities, was observed at the Coastal Laboratory, at Carmel, California, and may be briefly referred to in this place. Among the species growing in the experimental plots at the laboratory are *Opuntia versicolor* and *Fouquieria splendens* from the vicinity of the Desert Laboratory, Tucson, Arizona. Owing to the usual low temperature of the air, and soil, these species generally make little or no shoot growth at Carmel. When, however, the roots of the plants are kept in soil whose temperature is 25–30° C., the shoots remaining in the cool air, not only do the roots grow rapidly, but new shoots and fresh leaves are promptly formed. Without pursuing this phase of the matter further it can be seen that analogous results might occur in nature should the soil conditions, for instance its color or the relation of the soil surface to the incident heat rays,[1] be such as to bring about a relatively warm soil environment. Under such conditions it is clear that only a study of the soil temperatures, and the responses of the roots to soil temperatures, would provide the key to the solution of the shoot behavior and to all of its accompanying results.

It is generally recognized that the soil acts as a reservoir for heat, and that the daily course of soil temperature is unlike that of the air immediately above it. Thus, the roots are subjected to temperature conditions which are quite different from those affecting the shoot of the same organisms. The shoot is warmer by day and colder by night than the root and it is improbable whether the roots of most woody plants are often subject to "optimum" temperature conditions, as must frequently be the case of the shoots. An exception to this statement, however, is to be found in the cacti where the most favorable soil temperatures are of great importance among those environmental features that may be called definitive. The roots of most cacti of the Tucson region, and possibly elsewhere, lie near the surface of the ground. For the most part they are less than 30 cm. deep. Inasmuch as the rate of root growth of the cacti, as will be shown below, is relatively slow at temperatures much under the "optimum," the importance to these plants of a shallow position of the roots will be apparent. It is only in the upper soil horizon that such favoring temperatures are to be found. It is of inter-

[1] Cannon, W. A., "On the Relation of Root Growth and Development to the Temperature and Aeration of the Soil," *American Journal of Botany*, Vol. 2, p. 211, 1915.

est to note, on the other hand, that deeply placed root-systems, such as of *Prosopis velutina*, may have a relatively rapid growth rate at relatively low temperatures.[2] In such a case it is quite possible that the rôle played by root response to temperature in species distribution may be less important, or, at any rate, different from that played by the roots of the cacti, for example, to the distribution of members of that family.

We will now glance at the most striking conditions of soil temperature as they obtain at the Desert Laboratory, where much of the work here referred to has been carried on, before taking up a résumé of the response of the roots of the cacti to the temperature of the soil and the relation this suggests to the general distribution of the family.

Three series of soil thermographic records, which are now being supplemented by others, have been kept at the Desert Laboratory. These relate to three depths, namely, 15 cm., 30 cm., and about 2.6 m. Although the records cover a series of years, it will serve the purpose in hand if we refer to those for the year 1910 only.

The mean maxima and the mean minima temperatures for the three depths will provide sufficient data for interesting comparisons.

At the shallowest depth, 15 cm., the mean maxima temperatures for midwinter and midsummer were 8.1° and 34° C., respectively. The mean minima, for the same seasons, were 3.9° and 30.8° C. At a depth of 30 cm. the maximal range was from 12.2° C., in January, to 33° C., in July, and the minima temperatures, for the same months, 10° and 32.2° C., respectively. It was observed that from June to September, inclusive, the curve of the mean maxima for this depth did not fall below 32.2° C.

At a depth of 2.6 m., the mean maxima temperatures ranged from 18.6° C., in January, to 27° C., in July.

Upon comparing, in a general way, the mean maxima for the different soil depths we see that the shallowest soil is the warmest from April to August, inclusive; that in September and October only the highest temperatures are found at a depth of 30 cm.; and that in late winter-early spring the lowest level is also the warmest.

The relation of the rate of root growth in *Opuntia versicolor*, as representative of the cacti, to different soil temperatures indicates interesting conditions and possibilities, and will be given in the following paragraph:

[2] Cannon, W. A., *l. c.*

Very many experimental cultures, of various kinds, made both at the Desert Laboratory and the Coastal Laboratory, have shown that the growth rate of the roots of *Opuntia,* within limits, varies directly with the temperature. It is relatively slow at 20° C., and most rapid at 34° C. The hourly increase in length of the roots at 20° C. is about 0.3 mm., and at 30° C. it is approximately twice this. Above 34° C., the rate falls off rapidly and ceases at about 42.5° C. Below 20° C., the growth rate is very slow, as, for example, at a temperature of about 16° C. an increase in length of a perfectly normal root was found to be only 1 mm. in 14 hours. The maximum rate, taking place at about 34° C., is about 1 mm. an hour.

Referring back now to the soil temperatures, it will be seen that the roots of this species are exposed to optimum conditions in July and August only, although the soil temperatures for one month before and one month following this period, at a depth of 30 cm., or less, is also high enough for an effective growth rate. The soil temperatures, at this depth, in the other months, and at the lowest level throughout the year, are not sufficiently high for the best root activity. However this may be, we find, in short, that suitable soil temperatures obtain at the depths occupied by the roots of the cacti during four months of the year. But it does not follow that root growth goes on throughout this period for the reason that the foresummer is arid and the shallow soils are impossibly dry, having less than 10 per cent. of moisture. Active root growth of the cacti, in fact, commences with the coming of the summer rainy season, about the middle of July. It is ended by the cooling of the soil in early autumn. The length of the active growing season of the roots of the cacti, therefore, does not usually exceed six or eight weeks.

It is in the response of the roots to the temperature and moisture conditions, as just sketched, that lies the crux of the suggestion offered in this paper, namely, that conditions being otherwise favorable, the cacti, which are shallowly rooted, occur in such regions as have the superficial soils moist at the same time they are suitably warm, and they are wanting where such soil conditions fail.

With the reaction of the roots of the cacti to temperature in mind, it will be instructive to examine briefly the leading climatic features, so far as they affect the case in point, of the regions in which the cacti form a conspicuous portion of the vegetation.

According to Engler and Prantl, the cacti occur mainly in the

dry parts of Mexico, in the portions of the United States which border on Mexico, in eastern and central Brazil, and in portions of the Andes countries. Taking two or three genera as examples, we learn, for instance, that *Cereus* occurs in Mexico, and in the Andes of Argentina and Brazil. *Echinocactus* extends from the southwestern part of our country to Brazil and Chili *Opuntia* is found in Mexico, Peru, Chili, in Central America and in the southwestern portions, especially, of the United States. Although certain species are outside of this range, as especially certain opuntias, where the winters are exceedingly cold, all are subject in summer, when active growth takes place, to conditions which are in rather close accord. A glance at the summer climates of these regions will, I think, establish this point.

In the central part of Mexico, at Tehuacan, the annual rainfall is about 15 inches, most of which occurs in summer, and at Pueblo, 70 miles distant, and at a higher altitude, where the annual precipitation is more than twice that at Tehuacan, 72 per cent. of the rain comes in the warm season. The Tehuacan region has been characterized as being the richest of any known in cacti.[3] At Chihuahua, where the rainfall is 10.86 inches, the amount falling in the summer season is also over 70 per cent.

In the southwestern part of the United States, where the cacti constitute a conspicuous portion of the flora, a relatively large summer rainfall is also reported. At Tucson, for example, the precipitation amounts to 11.74 inches annually, of which 54.7 per cent. is received in July, August, and the first part of September.

Turning now to South America, and without especial regard as to the presence of cacti at the particular stations quoted, we find that over a relatively large area, a large percentage of rainfall is in the warm part of the year. For example, at Matto Grosso, Brazil, the greatest rainfall is in December. From June to August and generally for a month before and after this period, the climate is usually dry.[4]

Along the east coast rain occurs from February to April, June to September being dry. In the Cordilleras of Bolivia and Peru, the rainy period is in December–March, and the climate is dry from April to October. At La Paz, although rain may fall any month of the year, December to February is regarded as being the season of rain.

[3] MacDougal, D. T., "Botanical Features of the North American Deserts," Carnegie Inst. Wash. Pub. 99, 1908.

[4] Hann, "Handbuch der Klimatologie," Bd. II, 1910.

We have supplemental evidence that the cacti grow most successfully in such warm temperate moderately arid regions as have precipitation in the warm season from the work of the Australian commission for the study of certain species which have escaped from cultivation in several countries, especially Australia, and have become a pest.[5] In Queensland and New South Wales species of *Opuntia* constitute a serious weed. At Westward and Rockhampton, Queensland, where the cacti are particularly a nuisance, over 50 per cent. of the annual rainfall occurs in December–March, inclusive. Soil temperature data from Brisbane, depth one foot, show that the mean temperature from October to April is between 22.7° and 27.9° C., and that during the colder portion of the year the mean temperature at that depth is below 20° C.[6]

The commission studied the cactus problem in several different portions of the world, among which were Cape Colony, central and southern India, southeastern and southern South America and the Mediterranean region. It will be instructive to sketch the leading climatic features of definite localities where cacti were found to have escaped cultivation.

In southern Africa, species of *Opuntia* occur in a naturalized condition in the Great Karoo and in the Transvaal. In parts of the former region, as at Graaf Reinet, the species are abundant. At Graaf Reinet, according to Knox,[7] where the total precipitation is 15.29 inches, 63 per cent. occurs in November–March. In the Transvaal, where the escaped cacti are less numerous, the rainfall is 26.94 inches, of which 81 per cent. occurs in November–March.

In northern Africa the cacti escape from the oases very little, and the same is to a degree true of other portions of the Mediterranean region. In Algeria and Tunis, according to Knox, the rains are almost exclusively restricted to the winter season.

In India species are naturalized over a large territory, as, for example, in the Madras Province and in the Panjab. In Madras the prickly-pear has become a formidable evil throughout several districts. At Madras[8] 79 per cent. of the total precipitation takes place in August–September. In the state of Mysore, also, the

[5] Report of the Prickly-pear Traveling Commission, Brisbane, 1914.

[6] "Results of Rainfall Observations made in Queensland," H. A. Hand, 1914.

[7] "The Climate of the Continent of Africa," 1911.

[8] Hann, "Handbuch der Klimatologie," *l. c.*

opuntia is commoon. At Mysore, according to Hann, 81 per cent. of the rainfall is from May to October. At Lahore the prickly-pear is not so abundant as further south, but it occurs escaped, nevertheless. Here the July–August rains comprise 55 per cent. of the total annual precipitation.

In South America the Commission examined naturalized opuntias in portions of Brazil and Argentina chiefly. An important prickly-pear region is northwestern Argentina, where native as well as introduced species of cacti occur in abundance. At Salta there is as good as no rain in the cold season, between May and September. At Tucuman, 69 per cent. of the rainfall takes place between December and March, inclusive (Hann), and at Catamarca, between November and March, inclusive, 81 per cent. of the total annual precipitation occurs.

Without pursuing this phase of the matter further, it would appear, in short, that in regions where cacti are abundant, either native or introduced, rains occur during the warm season. It is not intended to discuss in this place the actual amount of rainfall which falling in the warm season makes the presence of a cactus flora possible. It is well known, however, that the amount of precipitation in regions where cacti occur is extremely unlike, and that it may vary from season to season in any one region. This last, in fact, is one of the leading characteristics of an arid, or semi-arid region. So far as regards the precipitation differences in separate regions frequented by cacti, it is interesting to note that at Rockhampton, Queensland, it is 40.09 inches,[9] while at Phoenix, Arizona, it is 7.06 inches,[10] and that in the former region 20 inches occurs in the warm season, while the amount of summer precipitation at Phoenix is between 0.9 and 2.1 inches, as means of the extremes.[11]

In the Mohave the annual rainfall is 4.97 inches,[12] about two inches less than the mean precipitation for Phoenix. In the Mohave, however, 86 per cent. of the rainfall is in winter, which greatly emphasizes the differences in summer aridity of these regions, and points to a probable reason why cacti are almost

[9] "Results of Rainfall Observations made in Queensland," H. A. Hunt, *l. c.*

[10] "Botanical Features of North American Deserts," D. T. MacDougal, p. 95, 1908.

[11] "Climatology of the United States," A. J. Henry, U. S. Dep. Ag. Bull. Q., 1906.

[12] MacDougal, *l. c.*

wholly wanting in the flora of the latter region. From these climatic facts it appears that while soil moisture is a condition *sine qua non* of the presence of the cacti, the range of the actual amount of soil moisture must be very great indeed, so, in short, it results that the temperature is the factor in direct control, thus a very important limiting factor.

Should we sum up, therefore, the factors thus far mentioned as being important among those which determine the distribution of the cacti, we find, in the first place, that the shallowly placed root-system subjects the roots to the greatest possible extremes in soil temperatures, including those that are high, and, at the same time, makes it possible for the plants to advantage from the minimum effective rainfall. Further, an effective growth rate of the roots takes place only at relatively high soil temperatures. And, finally, a certain but highly variable amount of moisture must be present in the soil. Since the *crux* of the matter, however, appears to be the fact that the root-system of the cacti are essentially superficial, there is the additional factor, or factors, which bring about this circumstance. These are at present unproved, but the results of experimental studies, not published, indicate that among them must be included the response to the oxygen supply of the soil.

W. A. CANNON

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THE INHERITANCE OF CONGENITAL CATARACT

IN the February number of the AMERICAN NATURALIST there is an article from the Bussey Institution by Jones and Mason[1] in which an attempt is made to show that congenital cataract behaves in heredity as a simple Mendelian recessive. The authors from a study of family histories published by Harman in the "Treasury of Human Inheritance" come to conclusions at variance with those of Bateson and Davenport, which authors they are perhaps unjustly disposed to criticize. The paper is well written and embodies a considerable mass of data, so that the reader not familiar with this particular problem might easily be led to think that the older investigators had really made a mistake in interpretation. The evidence, however, does not seem to

[1] Jones, D. F., and Mason, S. L., "Inheritance of Congenital Cataract," THE AMERICAN NATURALIST, Vol. L, No. 590, pp. 119–126, February, 1916.

warrant such a conclusion, as the present paper will attempt to demonstrate.

It is stated on page 120 of the article in question that the data used in the paper are taken from the tables accompanying Harman's publication. Since we are concerned wholly with a question of interpretation we may confine ourselves to these tables.[2] The families recorded in the tables are classified by Jones and Mason as follows (p. 120):

> After discarding all the doubtful cases, and picking a sibship with its parents from the table as a family, there is left a total of one hundred and twenty-five families which are classified into three different categories, as follows: (*A*) Both parents normal with at least one abnormal child; (*B*) one parent normal, the other affected with some form of congenital cataract, with at least one abnormal child; (*C*) both parents abnormal, giving only abnormal children.

In each of these groups (*A, B, and C*) it is thought that evidence is found in support of their contention that congenital cataract is a recessive character. We may now consider this evidence in the order in which it is presented.

In group *A*, 31 families are cited in which both parents are normal with one or more affected children. This is the strongest, or really the *only*, evidence that is offered in favor of the recessive character view. Let us examine it more closely. On going over Harman's tables, we find that of these 31 families there are 16 in which the affected individuals produced no offspring or nothing but normals. We do not wish to lay great emphasis on this point, but in such cases one should bear in mind the common clinical belief and the experimental proof (for rabbits and guinea-pigs)[3] that a certain number of congenital cataracts are produced by intrauterine poisoning without necessarily any reference to heredity.

Another possible explanation for some of the examples in group *A* is that they represent cases of origin *de novo*. Jones and Mason say:

[2] Harman, N. Bishop, "Congenital Cataract," in the "Treasury of Human Inheritance," Part IV, Section XIII *a*. Eugenics Laboratory Memoirs, XI, pp. 126–169. Pl. XXVIII–XXXIII. London, 1910.

[3] Pagenstecher, H. E. "Über eine Methode der gemeinsamen experimentellen Erzeugung von Augenmissbildungen und von angeborenen Staren bei Wirbeltieren," *Münch. Med. Woch.*, 58 Jahrg., No. 32, pp. 1716–1717. Aug. 8, 1911. (Reviewed in most of the eye journals.)

Surely it is not possible to explain so many cases as *origin de novo* or as due to faulty classification of the parents.

With reference to the origin *de novo* of characters it may be recalled that one does not have to search the literature long to find instances of the same mutation occurring repeatedly in different stocks and at different times, or of certain stocks that seem to be especially prone to mutation.[4] Congenital cataracts occur in many races of man and in other mammals. So far as the writer is aware we are not at present in a position to state, either on the basis of observed data or from *à priori* consideration just how frequently mutations may occur in the human germplasm.

Again, since Jones and Mason elsewhere in the same paper (p. 124) use the argument that "heterozygous individuals sometimes show the recessive character," we might, if necessary, use the same argument to prove the dominance of cataract. On the assumption that congenital cataract is dominant instead of recessive it might be maintained that in those cases where both parents of affected individuals seem to be normal, one of them is, after all, heterozygous, and affected children are therefore to be expected.

Finally it should be recalled that in their statistical study of these 31 families Jones and Mason do not get the results that their hypothesis demands. After having made the proper mathematical corrections there still remains a discrepancy which they do not adequately explain, the agreement between theoretical and observed results being only .418 (p. 122). In order to test what one should expect from the examination of such data when the character is recessive, I have taken a paper by Usher[5] on retinitis pigmentosa and summarized the charts in the same way that Jones and Mason summarize those of Harman. Now retinitis pigmentosa probably *is* a recessive character as is commonly believed. In the charts of Usher are recorded 44 families in which

[4] To cite a single case, we may mention the results of Barfurth in breeding fowls. In normally 4-toed races polydactylism occasionally arises *de novo*, but once having appeared is transmitted as a Mendelian dominant. Barfurth, Dietrich, "Experimentelle Untersuchung über die Vererbung der Hyperdactylie bei Hühnern. V. Mittelung: Weitere Ergebnisse und Versuch ihrer Deutung nach den Mendelschen Regeln," *Arch. f. Entwichlungsmech. d. Organism.*, Bd. 40, pp. 279–309, 1914.

[5] Usher, C. H., "On the Inheritance of Retinitis Pigmentosa with Notes of Cases," The Royal London Ophthalmic Hospital Reports, Vol. 19, pp. 130–236. 1914.

neither parent of the affected individual shows the defect (*i. e.*, both are presumably heterozygous). These 44 pairs of parents are recorded as having 320 children, of whom 77 are affected—24 + per cent. as compared with an expectation of 25 per cent. If the data on cataract were to yield results as close as this we would be more disposed to credit the view that the character is recessive.

So far as the 31 families of category *A* are concerned it must be admitted that absolute proof of the fallacy of the recessive character view can not be furnished, but it will be apparent that there is considerable evidence which not only fails to support this view, but actually points decidedly against it. This fact, taken in connection with the positive refutation which the data in categories *B* and *C* supply, makes a very strong case against the view that congenital cataract is a recessive character.

In the second category (*B*) where one parent is affected the other normal, Jones and Mason remark that "the number of affected children would be expected to be approximately the same whether the character was inherited as a dominant or a recessive" (p. 121). But it must be borne in mind that the offspring of a recessive show the 1:1 ratio *only* when the mate is heterozygous, and in their second table Jones and Mason assume that the parents of the children in group *B* represent the cross "Nn × nn." The question is not raised as to the probability of the occurrence of such matings nor does there seem to have been an attempt made to trace the offspring from the normal and affected members of the F_1 generation. In other words, the data of really critical significance do not seem to have been considered. As it stands, then, Table II seems to present no evidence either for or against the above hypothesis, a point which the authors themselves recognize as the quotation indicates.

Since the authors have not tabulated the data which would seem to be of most significance, we may return to Harman's original charts assuming for purposes of the discussion that congenital cataract really is a recessive. On this assumption there are two important conditions which we should expect to find fulfilled.

1. If congenital cataract were a recessive, a cataractous person married to a normal should in most cases produce only normal children. This will be apparent when it is recalled that con-

genital cataract is so rare (perhaps 1 in 4,000 or 5,000)[6] that the number of heterozygous individuals in the general population must be relatively low—theoretically not more than 1:30.[7] In other words, if congenital cataract were recessive the chances that an affected individual in marrying would get a heterozygous partner and thereby be able to produce affected children would be only one in thirty and the chances that the same thing would happen in several generations in direct descent *as occurs repeatedly in the charts* (the case in over 40 *different family trees*) become extremely remote. We should not then expect families with one cataractous parent to contain affected children more often than in the above proportion.[8]

2. If congenital cataract were recessive the normal children of a cataractous parent should themselves produce affected children in half as many cases as do their cataractous sibs and the total number of affected children produced should be one half as great in the first case as in the second. This expectation follows from the assumption that the original (grandparental) mating was Nn × nn. As a result of such a mating the F_1 generation can be composed only of Nn and nn individuals. Neither of these should produce affected children except when married to an Nn (or an nn), and the chances of such a marriage are as great (or as remote) in the one case as in the other. In other words, an equal number of heterozygous and pure recessive individuals of the F_1 generation should get heterozygous mates. In these few families the expectation for F_2 would then be of a 1:3 ratio for the Nn × Nn matings and a 1:1 ratio for the Nn × nn, which would obviously give one half as many affected children in the first case as in the second.

Harman's charts afford sufficient data to settle these points conclusively. In regard to the first point there are 96 cases in which the cataractous child of a cataractous parent has himself

[6] This statement is an estimate based on data gathered from a number of sources, *e. g.*, *Jour. Amer. Med. Ass'n*, quotations from the census reports, etc. It is probably not too low, but if the incidence were as much as 1:100 we should still find a similar but of course less marked discrepancy.

[7] This result is arrived at by the use of a formula similar to one given by Jennings in his paper "The Numerical Results of Diverse Systems of Breeding," in *Genetics*, Vol. 1, No. 1, pp. 53–89. Jennings is not responsible for the use of his formulae in this connection, but they obviously apply.

[8] Corroborative evidence on this point is furnished by the histories of retinitis pigmentosa in the paper by Usher already referred to.

reached maturity and produced one or more normal offspring, thus proving on the assumption that cataract is recessive, that his consort was either NN or Nn. Instead of finding, as we should expect on the assumption that cataract is a recessive, that only 3 or 4 (1:30) of these individuals would find an Nn mate and therefore be capable of giving some affected children, what we really do find is that of these 96 families, 83 have produced cataractous children—86 per cent. It is significant, incidentally, that of the remaining 13, ten are families of three children or less. A more striking refutation of the assumption could hardly be found.

In regard to the second point we find that there are 47 normal individuals in the same F_1 generation from the supposedly Nn $\times$ nn matings who have also come to maturity and produced children. In 42 of these families only normal children have resulted. In 10 per cent. of such families, however, one or more cataractous children have been produced. But the relation between 10 per cent. on the one hand and 86 per cent. on the other is very far from being the relation of one to two.[9]

These considerations based on the results from such matings as are included in category *B*, and the F_2 descendants of such matings, furnish convincing evidence that congenital cataract is not a Mendelian recessive.

Finally as to the last point, which concerns category *C*, we can agree with the authors when they say:

> The critical test as to whether or not congenital cataract can be considered as a simple recessive character lies in the matings of abnormal by abnormal. Families of this kind should have only abnormal children. Only three such matings are available.

I find two of these, but have searched the charts in vain for the third which must be an error or inadvertently drawn from outside sources. This really is immaterial since it would require many such cases to prove the hypothesis, where a single bona fide case in which two affected individuals produce normal offspring is sufficient to overthrow it. One of the three cases cited is such.

[9] How we are to explain the five individuals in this group who have produced cataractous offspring is not material to the present consideration. Not to mention the possibility of faulty classification, possible mutation etc., they might, for instance, belong to the class mentioned by Jones and Mason in which the parent while really heterozygous appears in the recessive form.

In chart 342, III–28 and III–37 are shown as a pair of parents both of whom are affected in both eyes. The descriptions quoted from Nettleship by Harman (*op. cit.*, p. 148) show that the diagnosis is evidently based on ophthalmoscopic examinations. This is a clear case of abnormal by abnormal, and if we were to regard it as "doubtful" we could find equal justification for so regarding any other chart in the whole series. The offspring of this marriage are seven children, of whom two have cataract, three thought to have been free from it died in infancy, and two are definitely known to be normal. This is the one critical case that is needed and, taken at its face value, it completely refutes the argument for the recessive nature of congenital cataract.

In conclusion, the writer does not wish to insist on arguments from a few particular cases, nor does he wish to make purely academic distinctions in the treatment of data. In particular, he does not wish to be understood as maintaining that congenital cataract behaves strictly as a single dominant unit character—a view to which he does not subscribe. The point upon which he does insist, however, is that the view, presented in the paper under discussion, namely that congenital cataract is due to a single recessive character, not only fails to find support in the data which was presented, but is in reality actually disproved by that data.

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THE FORM OF EVOLUTIONARY THEORY THAT MODERN GENETICAL RESEARCH SEEMS TO FAVOR

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Nature produces those things which being continuously moved by a certain principle contained in themselves arrive at a certain end.

—Aristotle.

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I. GENERAL STATEMENT OF THEORY OF EVOLUTION

THE history of evolution, all will agree, has been from the less specialized to the more specialized. The prevailing view is that this greater specialization has been achieved by adding qualities one by one to the less specialized until there has become built up through the ages so complex structures as the higher types of organisms. Organic evolution, from this view, has proceeded along the same lines as the evolution of an old English manor

house through the accretions of successive generations; or is like some medieval cathedral to which each generation has added some sculptures, some stones to the steeple, some new stained-glass windows. Organisms, the theory runs, began simple,—they varied, why or how no matter; variation is given and is in all directions. Positive variations toward greater complexity were usually advantageous and individuals showing such elbowed out of existence their less-favored cousins and established a new and higher level from which evolution might proceed.

This view has certain resemblances to an old view of ontogeny. The embryo is simple. Food is added and food makes this part and that grow. Why only certain parts grow in the presence of food and not all—the differential nature of growth—that is *given*. It is the nature of the organism that all parts should not grow equally; but the essential thing is that food and water and heat are the things that add parts and make the embryo larger and more complex.

This view of development has, I think it will be admitted, now become generally abandoned. To-day we recognize rather that the egg or embryo is not so simple, but that, on the contrary, it has wrapped up in it all of the potentialities that are eventually realized; potentialities that will be realized, however, only if conditions of life (food, water, heat, etc.) are appropriate.

The alternative view of evolution, like the modern view of embryonic development, lays more stress on the internal factors of evolution. It postulates that the primitive organisms, like the eggs, are not so simple as they look but have a molecular constitution of great complexity; and, just as the egg has a mechanism by virtue of which (under favorable conditions) it develops, so the ancestral protoplasm had a mechanism by virtue of which (under favorable conditions) it *evolved*. We do not know much about the specific molecular machinery that determines the specific nature of differential growth, but

we have reason to think that it is located chiefly in the nucleus. Similarly we are ignorant of the specific nature of the machinery that determines phylogenetic variations, but we have reason to think that it is located in the germ plasm and that the karyokinetic phenomena, especially the movements of chromosomes at and around the time of fertilization, have a great deal to do with such phylogenetic change. But as the egg with its given internal mechanism of development under adequate external conditions will develop into the specific adult form, so the primitive germ plasm with its internal mechanism of evolution under adequate external conditions has developed into all those forms or kinds of germ plasm that are responsible for the great variety of present and past organisms. Just as the egg-nucleus contains probably fewer kinds of molecules, but each more complex, than a nerve cell, *e. g.*, of the adult, so the ancestral form of protoplasm probably contained fewer kind of molecules each more complex than the derived forms. The derived forms, conversely, have more kinds each of simpler constitution.

In this view (if we may let our imagination picture the consequences) the foundation of the organic world was laid when a tremendously complex, vital molecule capable of splitting up into a vast number of kinds of other vital molecules was evolved! The capacity for thus splitting off molecules determines the possibility of production of the organic species with their vast number of characteristics.

It is to be kept in mind, however, that the number of genes is probably less than the number of elementary species. For numerous elementary species differ by only one or two genes and in many cases the species differ only in new *combinations* of the same set of genes.

This theory of evolution is not new; it is that briefly expressed by Bateson in his Australian address; it is very clearly expressed by Hagedoorn in Roux's "Vorträge" and has received the support of Lotsy (1913). It

is, however, essentially Nägeli's theory of evolution from within by virtue of a perfecting or progressive tendency, in support of which Nägeli himself draws the parallel between embryology and evolution. It has certain points of resemblance to Eimer's orthogenesis, but differs from it tremendously in that Eimer thought evolution was directed by the external world and that there were summed in the germ plasm the impressions of that world received in successive generations. Huxley was inclined to accept the theory of internal factors in evolution. He says:

> I apprehend that the foundation of the theory of natural selection is the fact that living bodies tend incessantly to vary. This variation is neither indefinite, nor fortuitous, nor does it take place in all directions, in the strict sense of these words. . . . A whale does not tend to vary in the direction of producing feathers, nor a bird in the direction of developing whalebone.

Mivart and many others have long contended that evolution is due to *internal* factors. Their views are in accord with Aristotle's.

Finally, I may close this section by quoting a simile from Bergson, as translated by Mitchell.

> The evolution movement would be a simple one, and we should soon have been able to determine its direction, if life had described a single course, like that of a solid ball shot from a cannon. But it proceeds rather like a shell, which suddenly bursts into fragments, which fragments, being themselves shells, burst in their turn into fragments destined to burst again, and so on for a time incommensurably long. We perceive only what is nearest to us, namely, the scattered movements of the pulverized explosions. From them we have to go back, stage by stage, to the original movement.
>
> When a shell bursts the particular way it breaks is explained both by the explosive force of the powder it contains and by the presistance of the metal. So of the way life breaks into individuals and species. It depends, we think, on two series of causes: the resistance life meets from inert matter, and the explosive force—due to an unstable balance of tendencies—which life bears within itself.

II. Support for the Theory from Collateral Fields

The view that the course of evolution (like the development of the individual) is chiefly determined by internal

changes receives support from various collateral fields of investigation.

1. First, from *embryology,* by analogy. The development of the embryo is directed from within. The process of development is one of specialization; a great number of tissues is produced but these tissues have lost the capacity, which the embryo has, of producing all kinds of tissues. Development is essentially an irreversible process just as evolution is, and for the same reason—that a fragment can not produce the whole. The adult individual is more complex than the fertilized egg, yet the egg has greater potentialities than any tissue-cell has. Regeneration depends on the presence of embryonic, *i. e.,* non-tissue, cells lying latent amidst the tissue cells. The greater complexity of the adult as a whole over the egg does not hold for a given tissue cell—*that* is *less complex* than the egg. The complexity of the adult is due to the fact that there are in the body many kinds of tissue cells, each simple, while the egg is just one kind of cell—but very complex in constitution. Similarly, we may infer that while the vast number of *kinds* of germ plasms in the higher organisms, differentiated in respect to their chromomeres, contrasts with the condition in the protista, it is probable that each chromomere of the protista is composed of much more complex organic molecules or molecule complexes.

2. Another mass of evidence for this theory is supplied by *paleontology.* By this science are offered extensive series showing: (*a*) the usual beginning of a new character as a simple, often inconspicuous trait, (*b*) the increase of variety in the course of evolution of a phylum ending in great outburst of extreme and bizarre forms immediately preceding the extinction of a phylogenetic line; (*c*) the irreversibility of the process of evolution; and (*d*) parallelism in evolution of allied lines.

(*a*) *The Simple Beginning of a Trait.*—The early history of a number of spinose groups of species shows (Beecher, 1898) that each group began its history in

small, smooth or unornamented species. The septæ of ammonites begin simple and later evolve their extraordinary foldings. The horns of titanotheres "have excessively rudimentary beginnings phylogenetically, which can hardly be detected on the surface of the skull" (Osborn, 1912, p. 253). Also, these "rudiments arise independently on the same part of the skull in different phyla at different periods of geological time."

(*b*) *The History of Progressing Phylogenetic Development.*—While paleontologists have no knowledge of germinal conditions they can study the course of evolution of a particular character through a lineage comprising thousands of generations. Paleontologists are agreed that characters tend to become more and more complex. So Beecher (1898, p. 354) writes:

> The smooth, rounded embryo or larval form [terms used in the phylogenetic sense] progressively acquires more and more pronounced and highly differential characters through youth and maturity. In (paleontological) old age, it blossoms out with a galaxy of spines, and with further decadence produces extravagant vagaries of spines. So in the titanotheres the horn rudiments evolve continuously, and they gradually change in form, . . . they finally become the dominant characters of the skulls, showing marked variations of form in the two sexes.

The saber-toothed and other tigers gained canines that they could not use. The mollusc, *Hippurites,* gains a shell a foot thick. In the Labyrinthodonts the infolding of the teeth has been carried to an extraordinary degree, etc. These are illustrations merely of what is said to be a general rule. F. B. Loomis[1] writes of it under the head "Momentum in Evolution."

(*c*) *The Irreversibility of the Process of Evolution* has been often remarked upon. It leads to exaggerated developments in one direction; lateral and backward variations are relatively uncommon.

Thus, the horse has shown divergent lines of evolution but none returning to the four-toed ancestral type. The ammonites went from simpler forms of septum to more and more complex without reversal, except at the very end of their phylum. D. Rosa says:

[1] AMER. NAT., 39 (1905).

An organ which in the course of its phylogenesis once disappears has disappeared for ever. . . . Not an exception is known to the rule.

Even an organ once rudimentary, like flying in some ground birds, never returns to full activity. A striking example of dropping out is that of cilia in Arthropods; which, ubiquitous in other groups of animals, are in this group gone throughout; they fail to develop even in spermatozoa. Toward the end of the process of evolution, a character tends to break up into a great number of new characters. These usually affect a certain organ, like the suture of the ammonites, and precede extinction of the phylum. We may say that the number of genes has become, through fractionation, so great that the resulting complexity or the resulting extremes of development of a trait are prejudicial to the development of the organism.

(*d*) *Parallelism of Evolution in Allied Lines.*—This parallelism was recognized by Darwin who writes:

The principle formerly alluded to under the term of *analogical variation* has probably in these cases often come into play; that is, the members of the same class, although only distantly allied, have inherited so much in common in their constitution, that they are apt to vary under similar exciting causes in a similar manner; and this would obviously aid in the acquirement through natural selection of parts or organs, strikingly like each other, independently of their direct inheritance from a common progenitor.

Of such parallel variations Osborn (1915, p. 216) speaks as follows:

Similar rectigradations may arise in all the descendants of similar ancestors at different periods of time; they always give rise to parallelism or convergence between the members of related phyla.

Illustrations of such are afforded in many paleontological monographs.

3. Another line of evidence for the theory of the primacy of internal factors of evolution is found in *experimental breeding*. This evidence appears in the facts (*a*) that many mutations begin small and can be rapidly evolved into highly developed characters, (*b*) that similar variations appear in related organisms, (*c*) that

mutation is limited to certain lines and (*d*) that experimental evolution seems *chiefly* due to dropping out of genes.

(*a*) That *characters often arise as rudiments* and only in the course of generations realize their full potentiality is a well-known experience of breeders. Thus De Vries[2] states that the double *Anemone coronaria* was produced by the owner of a nursery who, observing in his beds a flower with a single broadened stamen, saved its seeds separately and in the succeeding generations procured beautifully filled flowers. By appropriate matings Castle succeeded in 5 generations in getting much better expressed polydactylism in guinea pigs than he had at the outset. Dr. F. E. Lutz (1911) found a slight abnormality of venation in the fruit fly, *Drosophila ampelophila*. By in-breeding abnormally veined flies and selecting as breeders the extremely abnormal flies he eventually secured in the later generations some highly abnormal individuals. From a hen that showed only a slight extension of the web between certain toes I succeeded in breeding a race of profoundly syndactyl descendants.

(*b*) That *mutations* (*"saltations"*) *run in parallel lines* in related species is well brought out in a table given by Osborn (1912, p. 191), which I reproduce here with certain modifications.

That each germ plasm can vary only within certain limits and that related germ plasms show only a limited number of variations and the same in the different species indicate that variations of the specific rank are not determined by anything outside the organism, but by the very nature of the organism. Thus, the rabbit shows in its coat color the agouti coloration, and so does the guinea pig. From this in the guinea pig have arisen yellow, chocolate, black, albino and other colors; and a similar series has been obtained for rabbits. The guinea pig has produced an angora coat and so has the rabbit,

[2] "Species and Varieties," p. 491.

COMPARATIVE TABLE OF SALTATIONS

	1	2	3	4	5	6	7	8	9	10	11
	Man	Horses	Cattle	Sheep	Deer	Pigs	Dogs	Cats	Rabbits	Guinea Pigs	Mice
1. Proopic brachycephaly, abbreviation of face	..	..	×	..	..	..	×	..	..	..	..
2. Sudden development of horns on hornless races	..	×	..	..	..	..	..	..	×	..	..
3. Absence of horns on horned races	..	..	×	×	..	..	..	..	..	..	..
4. Jaw appendages	×	..	..	×	×	..	..	..	..	..	..
5. Taillessness, absence of caudals	×	×	..	×	..	..	..	×	..	×	..
6. Short-leggedness, or limb abbreviation	×	..	×	×	..	..	×	..	..	..	..
7. Consolidation of paired hoofs, syndactylism	×	..	×	..	..	×	..	..	..	..	..
8. Polydactylism	×	×	×	×	×	×	×	×	..	×	..
9. Epidermal thickenings	×	..	×	..	..	..	..	..	..	..	×
10. Mottled skin markings	×	×	×	..	..	×	..	..	..	..	..
11. Excessive hairiness, or length of hair	×	×	×	×	..	..	×	×	×	×	..
12. Hairlessness, entire absence of hair	×	×	×	..	..	..	×	..	..	..	×
13. Excessively fine or silky hair	×	..	×	×	..	..	..	×	×	..	×
14. Reversed hairs	×	..	×	..	..	..	..	..	..	×	..
15. Curled-hair	×	×	..	×	..	..	×	..	..	..	..

the cat, the dog, the goat, the sheep (Lincoln) and others. So in different species of *Drosophila,* I am informed by Dr. C. W. Metz, the same mutations occur—of course, without any relation to environment.

In a strain that has produced a mutation once we are apt to find the same mutation a second time. Thus De Vries states that the peloric toadflax does not set seed in nature, yet it occurs repeatedly in a given locality and even in distant localities. We conclude there is something in the structure of the germ plasm of the toadflax that permits a wholly useless, and indeed not naturally perpetuated, mutation to occur easily. Similarly, doubling of the floral parts occurs again and again in wholly unrelated species; often combined with complete sterility. Again, poultry sometimes show a great extension of the web between the toes. This occurs chiefly between digits III and IV, exactly where the syndactyl web occurs in man and where it occurs in wading birds that have only a single web between the toes—of which 8 genera might

be named. So far as I know, a marked extension of the web between digits I and II has never been observed; between II and III it is relatively rare; again, between IV and V it has, so far as I know, not been noted in mammals.

(*c*) *Variation is Not Indefinite and Multifarious.*—The case of syndactylism in poultry well illustrates the general principle of the *limitation* of mutation to particular narrow lines; and this is commonly the case. Thus, Dr. J. A. Harris has examined himself and with the assistance of others over 1,000,000 bean seedlings, and while extraordinary variations have been found, yet in the later hundreds of thousands no new ones have appeared; nevertheless, the possibilities in leaf form, variegation, etc., if we may judge from books on plant teratology, are by no means exhausted. And when we contemplate the variety of form assumed by first leaves in different species, is not the constancy in the form in beans striking evidence of the narrowness of variation and its restriction to certain lines?

(*d*) *Evolution by Loss of Genes.*—Finally, it has long been recognized that an extraordinarily large proportion of the mutations we meet with are recessive to the wild type. Castle has noted this of rabbits and guinea pigs[3] and Morgan has noted it in the case of *Drosophila*. In my work with poultry I was not impressed with it, as taillessness, polydactylism, syndactylism, white color of the Leghorn, rose comb, etc., are dominant mutations. Still, other genetics work (Baur, Shull and many others) is strengthening the conclusion that current evolutionary changes under observation are chiefly due to the dropping out of genes, and this supports the theory that evolution is proceeding largely by the loss of genes. However, the fact of dominant mutations can not be derived. Take, for instance, foot abnormality. Here a disturbing factor has appeared in the organism that was not there before. There is no *a priori* reason for doubting that the break-

[3] "Heredity," p. 86.

ing up of one of the genes that makes for the normal foot might have a residuum that positively interferes with the operation of other foot-forming factors.

The genetical studies also accord with other evidence as to the general irreversibility of evolution. We see that when a population contains only a recessive trait it is impossible by breeding inside that population to get back the dominant allelomorph. From a pure blue-eyed population we do not get back the primitive brown-eyed condition.

It might be thought that if evolution proceeds chiefly by loss of factors it would tend to simplification rather than increase of the number of factors. It seems probable, however, that the loss is not merely of a whole gene, but of some part of it; a fractionation, as it were, by which the gene becomes altered or split up into two or more. For example, it is probable that in man the loss of a gene (or a part of one) releases special nervous states, as, for instance, that in which musical combinations run through the brain, or numerical relations are rapidly worked through, etc. Thus the *general* result of experimental work in genetics gives support to the view of evolution by loss of genes and by their fractionation.

4. *Evidence from Evolutionary Changes in the Inorganic World. Radiation Studies.*—The view that evolution is primarily by internal changes receives unexpected support from the recent discoveries concerning the evolution of the elements. It is now well known that the element, uranium, under certain conditions of temperature, etc., undergoes a spontaneous change into ionium, ionium into radium, radium into polonium, an essentially lead-like substance. Similarly, thorium passes through mesothorium and radiothorium. Indeed, in a particular series, side branches may be given off; thus polonium is not derived directly from radium, or from radium C, but from one form of radium C called radium C_1. Indeed, "radiums" of different sorts, called radium A, radium B, radium C, are recognized and the atoms of these differ

from one another by, probably, one electron lost from each successive stage of the series. The series is thus shown graphically by Sieveking (1913):

$$\mathrm{Ra} \rightarrow \mathrm{Em} \rightarrow \mathrm{Ra}A \rightarrow \mathrm{Ra}B \rightarrow \mathrm{Ra}C_1 \nearrow \mathrm{Ra}C_2 \rightarrow \mathrm{Ra}D \rightarrow \mathrm{Ra}E \rightarrow \mathrm{Ra}F$$

III. Certain Consequences of the Theory

1. The acceptance of this theory requires a special *explanation to account for adaptation.* Eimer's theory of orthogenesis posited the direct action of environment on the germ plasm, a view which wider knowledge of facts does not support. It follows naturally from the hypothesis that new traits bear, at first, no relation to environment any more than the polonium that is derived from the uranium does. Darwin recognized that variations were not necessarily adaptive in their origin; also that it was not necessary that they should be adaptive in order to survive. Darwin says:[4]

> We clearly see that the nature of the conditions is of subordinate importance in comparison with the nature of the organism in determining each particular form of variation.

How then is adaptation brought about? Strictly, we may say adaptation is not the thing that is brought about, but rather absence of non-adaptiveness. Such adjustment as we find is, doubtless, only such a residuum of variants as has not proved incompatible with conditions of existence. Two kinds of variations may survive: (*a*) Those not incompatible with the conditions of the present environment and (*b*) those which, while incompatible with present environment, are not incompatible with some other environment into which the species may migrate.

2. *Relation to the Rôle of Selection in Evolution.*—There is going on to-day a great discussion as to the importance of selection in evolution. How does the matter look from the standpoint of this theory.

First, all are agreed that nothing has importance for

[4] "Origin of Species," p. 9.

evolution of the race except what modifies the germ cells, because they are all that goes from one generation to the next. However, we learn little or nothing about the potential traits of the germ cells by looking at these cells. Our knowledge of their hereditary composition depends upon the traits shown by the individuals that develop out of them. We may infer the genotype by observing the phenotype. But the phenotypical condition of a person is a more or less imperfect index to the genotypical condition of that person. The soma is an imperfect index to the germ plasm. The difference between the two schools, one asserting, the other denying the value of "selection," is based primarily upon the reliance placed on the sufficiency of this index. Castle says, in effect, the somatic condition of my rats in respect to the coat-pattern is so good an index of their germinal condition that whenever I select rats for a quality of the pattern I am selecting them very closely for the corresponding quality of their germ plasm. Pearl says, in effect, in poultry the egg-laying capacity of the hen is hereditary; yet it is so poor an index of her germinal idiosyncrasies in this respect that an individual with the somatic characters of high laying is no more apt to have the genes for high laying than an individual with the somatic character of low laying. Pearl says to make progress I must select for breeders those which have proved their germinal quality by belonging to a race of high layers. If the various sisters and daughters of the hen are high layers, that is more important than the egg-laying performance of the one individual, merely.

It seems to me that the whole question is a pragmatical one. If the somatic condition of a trait is a good index of germinal conditions, in any case progress can be made by selecting the soma in that case; if the soma is an inadequate index, then little or no progress will be made by selecting the soma. We know this by breeding experience. If we select parents because of absence of pigment in skin or eye, we select also a germ plasm devoid of the

capacity for forming such pigment. If we select parents because they have medium-brown hair we get offspring with blond or golden or red hair even, and it would take a long time to get a pure brown-haired race by this method of selecting. If the trait that we are trying to improve is very sensitive to environment, then the somatic conditions will be a very bad index of the germinal; but if not sensitive, the somatic may be a good index of the germinal. Thus, since in the fruit fly the number of bristles varies closely with food conditions, selection of breeders merely on the somatic state will not lead to much, if any, genetic progress.

The question of the potency of selection in nature comes back to the matter of value of the soma as an index of the germ plasm. If blue eye color affords insufficient protection from tropical conditions elimination of the blue-eyed individual kills off blue-eyed plasm and with each death the race is rapidly purified. But there is a limit to the process because brown eyes are preserved equally whether the germ plasm does or does not carry the blue-eyed condition. As there are twice as many simplex as duplex brown eyes, the complete elimination of the blues will not be brought about quickly.

When, therefore, we hear a breeder say: "I made this character by *selection*" what he really means is: Somatic variations in the desired direction were afforded; there was a large correlation between somatic and germinal conditions, so that I was able, merely by choosing as breeders individuals showing the desired trait somatically, to get a race with the determiners of the character pure, or practically pure, in the germ plasm.

One other difference of opinion there seems to be between selectionists and the others. Castle evidently doubts if factors for characters are always discrete and do not change. He is inclined to hold that there may be genes which vary *pari passu* with a variation of a "unit character" and in somewhat the same degree. This view seems to be quite in accord with an expectation that

is based on experience, that traits shall be found the germinal bases of which are undergoing current evolutionary changes through loss of genes or through fractionation. And it is in accord with expectation that mutations shall reveal themselves just at the extreme of a series as Castle's plus mutation revealed itself. Others are inclined to think that the varied color pattern of Castle's rats is really determined by several factors (restrictors or extensors of the gene for "hoodedness") which were all in his race of rats at the beginning of his experiment. Upon one point all geneticists are, however, agreed—that we must interpret all of our results in terms of genes alone.

One other bearing of the orthogenetic theory deserves to be pointed out. If the germ plasm is capable of undergoing a spontaneous mutation which is the main source of evolutionary change, this fact would seem, at first blush, to indicate the futility of trying to control genetic change experimentally, except by the selection of germ plasms. Naturally, under these circumstances our effort would be limited to what nature affords. However, we do not yet know enough to put these limits on "experimental evolution." There is some evidence, although not as critical as might be wished, that the germ plasm is not beyond the reach of modifying agents. At least we must continue experimental efforts in that direction.

IV. Summary

A theory of evolution that assumes internal changes chiefly independent of external conditions, *i. e.*, spontaneously arising, and which proceeds chiefly by a splitting up of and loss of genes from a primitively complex molecular condition of the germ plasm seems best to meet the present state of our knowledge.

Such a theory receives support from various fields.

1. From ontogeny, where the differentiated end stage is derived from a relatively undifferentiated, but probably molecularly complex egg.

2. From paleontology, where the history of the phylum seems governed by internal laws.

3. From experimental breeding where progress is afforded only as internal changes permit.

4. From analogy, with evolution in the inorganic world, so far as may be inferred from the studies on the "rare earths."

Such a theory makes clear that success in "selection" depends on rate and amplitude of internal change and ability to judge of germinal from somatic conditions.

It renders less hopeful (but not hopeless) the prospect of being able to control completely by experimental methods evolutionary change.

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COMPARATIVE RAPIDITY OF EVOLUTION IN VARIOUS PLANT TYPES

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DURING the course of evolutionary development among the higher plants certain groups have evidently altered with such exceeding slowness as to retain their ancient constitution practically intact for very long periods; whereas others, by their more rapid accumulation of heritable variations, have during the same time undergone far-reaching changes and become developed into new and radically dissimilar types. These differences in the rate of evolution are apparently due in part to differences in mutability, in the extent to which hybridization has occurred, in the degree of diversity presented by the environment or in the keenness of the struggle which is waged for survival. The purpose of the present paper is to call attention to still another factor which seems to be of much importance in determining the rapidity of evolutionary change among plants, namely the length of the generation or period from seed to seed.

The time necessary for the attainment of reproductive maturity among plants is definitely correlated with the growth-type to which a species conforms. Trees are very slow to mature, some arriving at an age of eighty years or more before their first flowering time and very few indeed, under natural conditions, fruiting before the tenth season. The scanty available observations seem to indicate that the average period from seed to seed in arborescent forms is in the vicinity of twenty years. Among shrubs the generations are decidedly shorter, varying usually from three to ten years. The herb is the most rapidly maturing of all, a single year sufficing to develop seed in an annual and but two (rarely more) in a biennial or perennial. Most herbaceous species will thus have

from fifty to one hundred generations a century, most shrubby ones from ten to thirty and most trees only four or five. The degree of variability and other factors being equal, therefore, one would expect changes to accumulate much more rapidly and evolutionary progress consequently to be much faster among herbs than among woody plants. Is there evidence that this is actually the case?

In an attempt to obtain such evidence an analysis as to growth-habit was first made of the endemic portion of the floras of several regions. Upon the biological isolation of an area the new varieties, species and genera of plants which gradually take their origin in its flora are necessarily limited in their distribution to the region in question, or are "endemic" in it; and these local types will evidently be produced first and in greatest abundance by those elements in the flora which are changing most rapidly. Consequently that particular growth type which is found to predominate among such endemic forms may justly be regarded as the one whose members are undergoing the most rapid alteration. In an analysis of the endemic element in any flora, however, caution must be used to distinguish carefully two radically different types of endemic plants: those under discussion, which were local in origin and have never spread abroad; as contrasted with those which owe their present localization rather to the fact that they are isolated survivors of genera or species at one time much more widely distributed. The former category, which we may call the "indigenous" endemics, will evidently represent a new element in the flora; the latter, or "relict" endemics, a very old one. It is not always easy to separate sharply these two types in a given flora, but species, and especially genera, which stand apart and possess no near relatives in the region are, in most cases, at least, evidently to be looked upon as relicts; whereas in a group of species or genera the members of which are numerous and closely related one to another we doubtless behold a body of plants undergoing evolutionary development on the spot.

It is an analysis only of the latter type which is of significance for the present problem.

The genera of dicotyledons endemic[1] in temperate North America (Canada, the United States and northern Mexico) and in Europe (inclusive of the entire Mediterranean floral province) were studied in this connection. 400 genera were recorded as being endemic or essentially so in temperate North America. In this imposing array of types which are limited in their distribution to this region approximately 130 stand in isolated positions in the flora, being quite without near relatives, and are presumably "relicts." To this category belong *Carya, Planera, Maclura, Garrya, Sassafras, Xanthorrhiza, Baptisia, Nemopanthus, Ceanothus, Dirca, Dionaea, Hudsonia, Rhexia, Ptelea, Decodon, Houstonia, Symphoricarpos* and many other familiar plants. That these now exclusively American genera were indeed at one time much more widely distributed is indicated by the fact that many of them are found as fossils in Europe and Asia to-day. They undoubtedly represent a very ancient element in the flora. The most noteworthy feature of these "relicts" is that they include practically all the genera of *woody* plants endemic to this region, the typically American trees and shrubs.

Such ancient and isolated relicts, however, compose but a minority of the endemic genera. The remainder evidently belong to the other category and owe their endemism rather to the fact that they have been developed in America and have never spread beyond its borders. It is such endemic types which are of interest to our problem. That they are actually of local origin is rendered probable by their occurrence in groups of closely related genera (many of which are rich in species) each group presumably representing a separate center of evolutionary development and the nucleus for a new subfamily. There

[1] Genera in which 90 per cent. of the species or more are confined to the region in question were considered as endemic there. Genera rather than species were studied since they represent a greater degree of evolutionary change; and since endemism among species is so nearly universal on the two continents as to make its investigation of little value.

are over sixty such groups of allied genera in the endemic flora of North America. Notable among these are the alliances centering about *Eriogonum* in the Polygonaceæ; about *Streptanthus* and about *Lesquerella* in the Cruciferæ; about *Eschscholtzia* in the Papaveraceæ; about *Heuchera* in the Saxifragaceæ; about *Cercocarpus* in the Rosaceæ; about *Godetia* in the Onagraceæ; about *Cymopterus* in the Umbelliferæ; about *Pterospora* in the Pirolaceæ; about *Cryptanthe* in the Boraginaceæ; about *Trichostema* and about *Agastache* in the Labiatæ; about *Pentastemon* and about *Castilleja* in the Scrophulariaceæ, and about *Brickellia,* about *Solidago,* about *Boltonia,* about *Silphium,* about *Rudbeckia,* about *Hemizonia,* about *Baeria,* and about *Microseris* in the Compositæ. Most of these groups of genera have their center of distribution in the southwestern United States or in northern Mexico. The great majority of endemic genera in Europe are also evidently "indigenous" rather than "relict" in character. Centering chiefly in the Mediterranean region there are seventy or more groups of closely allied genera, notable among which are the alliances dominated by *Dianthus, Brassica, Alyssum, Lotus, Scandix, Asperula, Scabiosa, Anchusa, Anthemis, Carduus,* and *Cichorium.*

These "indigenous" endemic genera constitute a very characteristic and important part of the present-day flora of North America and Europe. Had they existed in anything like their present numbers and importance at the period when an easy exchange of plants was possible between the two northern continents, it is hard to believe that they would not now be well represented in the floras of both; particularly since herbs, of which we shall see these genera to be almost exclusively composed, tend more quickly than any other plant type to loose their endemic character because of the power to migrate rapidly and populate wide areas which is conferred by their ability to produce seed from seed in a single generation. This is well shown by the fact that in DeCandolle's famous list of 117 species, each of which at present occupies at least one half of the land area of the globe, there are none but her-

baceous forms. Since we have cause to believe that communication between North America and Europe existed until well into the Tertiary, the supposition is altogether reasonable that the genera in question have undergone at least the greater part of their development and dispersal since that time; and that in contrast to the "relicts" and the non-endemic genera they represent an element of very recent development in the floras of the two regions.

It is noteworthy that these indigenous endemic genera are composed almost exclusively of herbaceous species, *Cercocarpus* and its allies among the Rosaceæ furnishing practically the only exception to the rule in America. If the conclusion is correct that such endemic types are the most recently developed members of a flora, this dominance of the herb among them constitutes excellent evidence that it is the herbaceous element which has indeed been undergoing the most rapid evolutionary change.

In striking contrast to the highly endemic and local character of so many of the north temperate genera of herbs is the wide geographical range almost universal among the woody types. Nearly all the tree genera of temperate North America exist to-day on some portion of the Eurasian continent or give evidence by fossils that they once did exist there. The whole study of endemism in its relation to growth forms presents us with the picture of a very slowly changing woody vegetation, one which since the separation of the two northern land masses has given rise to few or no generic types, but which has been accompanied by a rapidly developing herbaceous flora so quick to originate new forms that upon isolation it has produced not only a throng of local species, but even a goodly number of genera.[2]

Evidence of value in the present problem may also be derived from a study of the relationship of the members

[2] The predominance of woody plants which is so pronounced in the endemic element of the floras of oceanic islands and of somewhat isolated continental areas in the south temperate zone, is evidently due to the fact that most of the herbaceous portion of the vegetation here has so recently arrived from its seat of origin in the great land areas of the north, that it has not yet had time to develop into endemic species and genera on a large scale.

of the various growth forms and their distribution in the modern system of plant classification. During the history of the vegetable kingdom, the continual evolution of species, genera and families has been opposed by their continual extinction, for one cause or another. One would expect that those types in which evolution was proceeding most rapidly would occur in groups, usually rather large, of closely related species and genera, and that members of such groups which subsequently came to be isolated by the extinction of their allies would soon become centers of development and give rise again to new groups. Monotypes would consequently be rare among them. The more slowly evolving forms, on the other hand, would be able to repair the ravages of extinction much less rapidly and easily and would therefore tend to occupy more or less isolated positions in the system, frequently as monotypic genera or families.

To determine the distribution of herbs as contrasted with woody plants in the present scheme of classification an analysis was made of the dicotyledons in Engler and Prantl's "Naturliche Pflanzenfamilien," supplemented and brought up to date as far as possible by the seventh edition of Engler-Gilg's "Syllabus der Pflanzenfamilien." The figures obtained of course can not be regarded as exact, but they are at least definite enough to bring out certain general facts.

One hundred and eight thousand species of dicotyledons were counted, grouped in 6,840 genera and 238 families; 4,030 genera, comprising slightly over 50,000 species, were found to be composed entirely of woody plants, and 2,630 genera, with slightly under 40,000 species, entirely of herbs; 180 genera, containing over 18,000 species, included both woody and herbaceous members and were disregarded in the count.[3] The average number of species in the woody genera is therefore 12.5, in the herbaceous ones, 15. In their large genera (including 10 species or more) the two

[3] If 90 per cent. of the species of a genus were woody, that genus was counted as "woody"; if 90 per cent. were herbaceous, it was counted as "herbaceous." "Mixed" genera include more than 10 per cent. of each type.

types are practically the same, there being an average of 45 species per genus in the former and 46 in the latter. This of course pulls the general averages together. The more scattering distribution of trees and shrubs, however, is made evident by the fact that of small genera (10 species or less) they possess 3,115, as compared with 1,890 for herbs.

The two types are somewhat more diverse in the number of genera per family. In an analysis to determine this distribution, only land plants of normal growth-habit were considered. The Balanophoraceæ, Rafflesiaceæ, Hydnoraceæ, Lennoaceæ and Cynomoriaceæ were excluded as aberrant; and families characteristically aquatic were also omitted, since from their uniformity of environment or other reasons unknown they are notoriously poor in generic types. Two hundred and twenty-four families of dicotyledons enumerated in the seventh edition of Engler's "Syllabus" remain to be considered; 130 of these are exclusively woody, 60 have both woody and herbaceous members, and only 34 are exclusively herbaceous,[4] making it evident at a glance that woody plants have much the wider taxonomic distribution. In the 190 families which include trees and shrubs, there are just 4,000 genera, an average of 21 per family. In the 94 families which include herbs, there are 2,590 genera, an average of 27.5 per family. (The 180 "mixed" genera were left out in this count.) The fact that the bulk of genera in both are massed in a few large families again pulls the averages together; but the more scattering distribution of woody genera over a large number of small families is decidedly emphasized when we note that there are no less than 39 monogeneric families of trees and shrubs, but only 7 of herbs (exclusive of aquatics). Of families with 5 genera or less there are among woody plants 83, among herbs only 24. In number of species per family the differ-

[4] Families like the Ranunculaceæ, Papaveraceæ, Crassulaceæ, Geraniaceæ and Umbelliferæ, which possess exceedingly few woody species, have been counted as strictly herbaceous; and others like the Sapindaceæ, Araliaceæ and Bignoniaceæ, in which there are very few herbs indeed, have been counted as strictly woody.

ence between the two types is still more marked, for the 190 woody families include about 59,000 species, an average of 310; the 94 herbaceous families about 49,000 species, an average of 510.

It is therefore evident that herbs tend to be massed in a comparatively few large genera and families. Among trees and shrubs, on the other hand, although the majority of species are also naturally in large, successful groups, there is a very much greater proportion of small genera and especially of small families, widely scattered throughout the whole taxonomic range, which have been isolated by the wholesale extinction of related forms and are apparently very slow to develop into larger aggregations. Such evidence as this, like that derived from a study of indigenous endemism, seems to indicate that the rate of evolution among herbs is decidedly higher than among woody forms.

There are doubtless numerous exceptions on both sides. *Cratægus, Eucalyptus, Acacia* and other genera of trees and shrubs appear to be rapidly developing new species, whatever the cause thereof may be; and many herbs, far from producing new forms, show every indication of being stationary or even of becoming extinct. These are both evidently exceptions to the general rule, however.

Of course these cases introduce the consideration of another factor, naturally thought of first in connection with rate of evolution among any organisms, namely, their comparative "variability," using the term in its broadest sense. To contrast members of the two growth types as a whole in this respect is necessarily very difficult, but an attempt was made to do so by comparing the proportion of varieties and named forms among the woody plants with that among the herbs in a number of floras. The results are shown in the table (p. 474).[5]

The proportion of varieties and forms is therefore practically the same among woody plants as it is among herbs, and if this is to be regarded as at all a criterion of variability, there is little to choose between the two growth

[5] Dicotyledons only are considered.

	Species		Varieties and Forms	
	Woody	Herbaceous	Woody	Herbaceous
N. E. United States....	532 (23%)	1,748 (77%)	144 (31%)	322 (69%)
Ceylon...............	1,123 (63%)	670 (37%)	165 (60%)	112 (40%)
Australia.............	3,970 (70%)	1,741 (30%)	752 (70%)	325 (30%)

forms. The greater plasticity probably belongs to the herb, owing to the larger independence of environment which its discontinuous existence allows. Brevity of life-cycle, however, rather than higher variability, is probably the cause of the more rapid rate of change exhibited by members of this type.

The conclusion that herbs have been evolved much more rapidly than trees or shrubs has certain important corollaries. There are at present approximately 2,600 genera of dicotyledonous herbs and 4,000 genera of woody plants. The much wider taxonomic range which we have noted in the latter type makes it probable that in extinct species and genera their numerical superiority is still greater. If herbs are thus decidedly fewer than woody plants in number of species and genera, but are nevertheless being produced at a much more rapid rate, it is highly probable that the herbaceous element in the flora of the world must have had a shorter evolutionary history than the woody one. This is in harmony with a considerable body of evidence derived from a study of the history, structure and distribution of the Angiosperms, which indicates that the most ancient members of the group were woody and that herbaceous vegetation has made its appearance in comparatively recent geological time.[6] The great steps in the evolution of the higher plants seem to have taken place while trees and shrubs were the dominant growth types, for all the important orders and the great majority of families are still composed wholly or in part of such plants. Herbs occur to-day in less than half the families of the dicotyledons, and although such a dominant portion of the vegetation in many regions, they seem to be of relatively recent appearance.

[6] Sinnott, E. W., and Bailey, I. W., "The Origin and Dispersal of Herbaceous Angiosperms," *Annals of Botany,* XXVIII, 1914, pp. 547–600.

A study of the comparative rate of evolution among the Angiosperms also throws a little light on the antiquity of this great group. Its fossil representatives have as yet failed to make their appearance in strata lower than the lowest Cretaceous, and it is commonly assumed that they had their origin at about that period. We have already noted the probability that they were represented at their inception only by woody forms. There is evidence that the herbaceous type, save perhaps as a negligible portion of the flora, did not arise till the early Tertiary, since which time its members have undergone practically their entire evolutionary development. As to the relative lengths of geological periods there is no very definite evidence, but authorities agree that the Mesozoic was of considerably longer duration than the Tertiary, a conservative estimate placing the former at about three times the length of the latter. Let us grant for purposes of argument that this is a reasonably close approach to the truth; and let us also assume that herbs have evolved at a rate averaging twice as great as that of woody plants, surely a moderate supposition. All the 2,600 exclusively herbaceous genera presumably developed as herbs; and there are 4,200 genera which contain woody plants and which probably originated as woody plants. Now if these 2,600 herbaceous genera have been developed since the beginning of the Tertiary, woody plants, producing new forms at half this rate, would during the same time have given rise,let us say, to 1,300 genera. To produce the other 2,900 woody genera (to say nothing of the hundreds which have become extinct) would require, assuming the same rate of evolution, another period twice as long as the Tertiary, thus thrusting back the origin of the Angiosperms to a date distant from the present thrice the length of the Tertiary. According to the estimate mentioned above, this would be about the beginning of the Jurassic. Of course this is all extremely hypothetical, but it serves to emphasize the probability that in order to have developed their great number and diversity of slowly changing

woody forms, the Angiosperms must have been in the process of evolution for a period many times as long as that since the origin of herbs, evidently beginning at a date far earlier than that at which the first angiospermous fossils occur.

This conclusion is still further strengthened by other facts. The earliest fossil Angiosperms seem to have been trees, presumably the most slowly alterable type of all. Furthermore, whatever may have been the importance in recent times of hybridization through cross-fertilization by insects as a cause of accelerated evolution, this factor was evidently inoperative at the origin of the Angiosperms, since, according to Handlirsch, flower-loving insects did not make their appearance till the Tertiary. The specialized character and apparently high phylogenetic position of many of the earliest fossil Angiosperms also renders it highly probable that they were the product of a long evolutionary history.

Evidence from all sources therefore seems to agree that the origin of these higher seed plants took place at a time very much earlier than their paleontológical record indicates. That they were not preserved as fossils in horizons lower than the Cretaceous is perhaps due to the fact that the earliest Angiosperms, as Professor Bailey and the writer have suggested,[7] appeared under essentially temperate climatic conditions, which in the Mesozoic were mainly confined to upland regions where fossilization would take place much less commonly than in lowlands. This apparent predilection of these primitive Angiosperms for an environment cooler than the tropical, together with what we have noted as to their high antiquity, suggest that the refrigeration of climate which took place in the Jurassic might have been a factor in their origin; and even tempts one to look farther back toward the epoch of markedly low temperatures subsequent to the close of the Paleozoic, which caused so many radical

[7] Sinnott, E. W., and Bailey, I. W., "Foliar Evidence as to the Ancestry and Early Climatic Environment of the Angiosperms," *Amer. Jour. Bot.*, II, 1915, pp. 1–22.

changes in the organic world, as perhaps the time when the angiospermous stock began to be differentiated from its gymnospermous ancestors.

We may point out, in conclusion, that a recognition of differences in the rate of evolution between various growth forms is evidently of importance in many problems concerned with the phylogeny, ecology or distribution of the higher plants. Upon the question, for example, as to whether the boreal species in the floras of southern South America and Australasia are of ancient or recent arrival there, some light is thrown by the fact that these species are almost all herbs. That so many members of a growth-form which is usually subject to rapid change still maintain specific identity with distant northern types argues strongly for their comparatively recent arrival in the south. A similar question is raised by Willis's studies of the flora of Ceylon.[8] He regards the endemic element here as one which is of local and recent origin and as much younger than the non-endemic element; but the fact that of the endemic species, by implication the ones which are changing the fastest, less than one fourth are herbs, whereas nearly one half of the non-endemic species belong to this growth-form, would suggest the opposite conclusion; for had this large body of herbs been in existence here for a very long period, as Willis supposes, we should have expected it to develop a relatively much greater body of endemic species. In such questions as this it should be borne in mind that evolution has not been a steady and uniform process among all forms, but that the decided differences between the various growth types in the rapidity with which they tend to accumulate heritable variations introduces a factor which it is always necessary to consider.

Summary

1. The most recently evolved element in the floras of temperate North America and of Europe, as determined

[8] Willis, J. C., "The Endemic Flora of Ceylon, with reference to Geographical Distribution and Evolution in General," *Phil. Trans., B*, CCVI, 1915, p. 307.

by a study of the indigenous endemic genera, is composed almost entirely of plants which are herbaceous in habit.

2. Herbs tend to be grouped in fewer and larger genera and families than woody plants.

3. It is therefore concluded that herbaceous plants, presumably because of the brevity of their life cycle and the rapid multiplication of generations consequent thereto, are in most cases undergoing evolutionary development much more rapidly than are trees and shrubs.

4. From this conclusion are drawn inferences as to the origin of the herbaceous habit and the antiquity of the Angiosperms.

EGG PRODUCTION AND SELECTION

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ABOUT three years ago breeding for increased egg production was begun at this station with Rhode Island Reds. A report on some of the chief features of the work will be published in the near future, but because of the recent discussion in this journal, by Pearl and Castle, of egg production in relation to selection certain features of egg production in this breed are of particular interest at this time.

The winter record of a hen depends upon two main internal factors aside from possible environmental factors. First, the date at which the first egg of a pullet is produced, which may be taken as an index of the attainment of sexual maturity; and second, the rate of production after the onset of egg-laying. The former in turn depends upon the time when she was hatched and upon her rate of growth. The latter factor, *i. e.*, rate, is controlled in part at least by an internal mechanism. In the Reds it is clear that of the two factors the preponderant effect is exercised by the factor of maturity. The observed differences in rate of production are of less, though by no means negligible importance. In the Barred Rocks, as far as I have been able to learn, conditions are reversed, in that the rate of production appears to exercise a greater influence on the kind of record a hen makes than the age at which she produces her first egg, although the latter is also a factor. Identical records as expressed in number of eggs per unit of time may therefore result, but it is clear that they are not directly comparable. The use of the age at first egg as a criterion of sexual maturity involves some difficulties, but it is the best objective criterion readily available and corresponds at least roughly with the general

bodily conditions familiar to poultrymen as indications of the attainment of maturity. These conditions may be observed in the male as well as in the female. There is also an apparent tendency on the average for large pullets to make lower records than their smaller sisters, as shown by the negative correlation between high winter egg production and large size, due probably to a longer growth period in the larger pullets.

The relation between age at first egg and rate involves the following points: A pullet that matures late can not give expression to high fecundity, although she may carry the genes for it, but the record of a pullet that matures fairly early will depend upon the rate at which she lays. On the other hand, a zero producer will be also late maturing, judged according to the criterion suggested, because she does not lay until spring, provided, of course, that she was hatched at the proper season. It is obvious of course that a high record bird must mature early in order to make her record, and that in this sense early maturity and high productiveness will go hand in hand, but early maturity of itself does not insure a high record, for early maturing birds may lay poorly and so make a low record. On the other hand, late maturity is bound to insure a relatively low record. In the flock of Reds with which we have had to do there is *comparatively* little difference in the rate of production. In one flock the coefficient of correlation for the time elapsing between the first egg and the 1st of March and the number of eggs laid was calculated and found to have a value of $.8612 \pm .0132$ which must mean that the flock was fairly homogeneous in respect to rate of production. In other flocks there is clearly a negative correlation between age at first egg and winter egg production but the values have not yet been determined. The difference between a pullet that begins to lay December 1 and lays 60 eggs, another the first of January and lays 40 eggs, another that begins the first of February and lays 20 eggs, must be a question of start. The last bird, too, must be essentially different from a

bird that begins to lay in November and lays 2 eggs that month, 10 in December, 0 in January and 8 in February, although she lays the same number of eggs as the individual that began February 1. The former is clearly a mediocre producer, the latter a high producer of late maturity.

The flock averages for the past winter, arranged according to the month in which the birds were hatched, are instructive on this point. The mean egg production for the (140) March-hatched birds was 39.8 eggs, for the (172) April-hatched 29.81, and for the (158) May-hatched 18.1 eggs. If the pullets be grouped according to Pearl's classification of zero, 1 to 30 (mediocre), and over 30 (high), there were among March-hatched pullets 6.4 per cent. zeros, 30.0 per cent. mediocre, and 63.6 per cent. high; among the April pullets 11.6 zeros, 37.3 per cent. mediocre and 51.1 per cent. high; while among the May-hatched pullets there were 19.9 per cent. zeros, 58.8 per cent. mediocre and only 21.3 per cent. high. The average winter production for the mediocre and high producers respectively, is for March-hatched birds 17.2 and 54.6; for April 16.6 and 46.2; for May 16.1 and 40.7. In this connection it should be recalled that there is a tendency for the abstract numbers involved to yield similar averages, which are an indication to some extent at least of two genotypes rather than one. Clearly, the time of year during which a Rhode Island Red pullet is hatched plays an important part in determining the class in which a given individual falls.

In general the use of a hen's record expressed in number of eggs per unit of time as the sole criterion of her capacity for egg production seems to us to be essentially wrong. Attention should also be directed to the elements that enter into the make-up of the record. The factor of maturity is not wholly a fecundity factor, though it is closely bound up with egg production and may modify a pullet's fecundity record very considerably. Broodiness is another factor that, although closely associated with

egg production, is after all rather of the nature of a separate entity which may or may not be present, but which when present operates to reduce the egg record of a hen very materially. On the average, a hen's record for the broody months is only about 60 per cent. of the same hen's record for the non-broody months. There is always a sharp break in monthly egg production with the onset of the first broody period and as a hen once broody continues as a rule to alternate brief periods of production with broody periods, it follows that the number of eggs laid depends in a large measure upon the number of broody periods.

Our viewpoint in regard to modifying factors for egg production may be observed in morphological as well as physiological characters. Thus, the color, arrangement and length of hair could not be studied in a hairless race, although the genes for color, arrangement and length may actually exist in the germ plasm of the hairless individuals. Variability in part at least is due to the existence of numerous germinal factors which can come to somatic expression only when some other germinal factor is present. Thus, there are several types of rose comb which appear to be due to definite factors that affect only the rose comb. The spike, for example, may be telescoped into the body of the comb. The telescoped condition is clearly inherited but it does not appear in singles of the same family, although there is no obvious reason why the blade should not be telescoped quite as readily as the spike. Before the rose comb can be said to be understood, the various genetic modifying factors involved, their relations to each other, and the mode of inheritance of each must be made out. Any study of selection that fails to take into account such internal modifying factors is incomplete.

There is no question regarding the inheritance of egg production in the sense that certain families make much better records than some other families, though in the Rhode Island Reds the families that make the better

records also mature at an earlier average age than those making poor records. Whether or not the mode of inheritance of fecundity in Rhode Island Reds follows Pearl's theory is still uncertain. Since there is great variability in the age at which the first egg is produced and since its frequency polygon indicates, at least up to 305 days, the essential homogeneity of the flock, we are inclined to believe that the Reds are unfavorable material for a solution of this question. More work, however, remains to be done before this point can be cleared up.

On Pearl's theory the continued selection of genetic high producers and the continued use of their sons as breeders should in the long run tend toward an improved egg production, even though individual pedigrees are not kept, as he himself has pointed out.

The rate at which the selection becomes effective depends much on what classes are assumed to be available at the start. In the following discussion it is assumed that the matings are made on a scale sufficiently large to permit the various matings to take place in the proportions indicated. The scale required, however, is so large that it would be scarcely practicable to test the matter experimentally.

In the first place, it should be pointed out that of the nine genetic classes of males possible on Pearl's theory, classes 5, 6 and 9 are not produced by any mating of class 1 and 2 females—the only classes laying 30 or more eggs—with any class of males. Moreover, it can be shown that if these three classes be assumed to be the only males available and that if only class 1 females ($l_1 L_1 . L_2 l_2$) be bred throughout the test and if the male offspring be bred in each generation in the same proportion in which they were thrown by the preceding generation, at about the seventh generation there would be male classes 1, 3 and 7 only, existing in the proportion of 1 : 2 : 1. Classes 2, 4 and 8 would indeed be present, but the three classes together would amount to only a little over 1 per cent. of the population. This small percentage decreases one half

in each succeeding generation. The females produced by this group of males mated to class 1 females only, would occur in the proportion of "over 30" females 75 per cent., "under" 25 per cent. The ratio 1:2:1 among the males maintains itself after once reached, so long as class 1 females only are used for mates.

The same ratio is reached if it be assumed that all nine classes of males exist in equal numbers at the start and that they are bred in each generation in the proportions thrown by the preceding generation and only to class 1 females.

The assumptions made have been chosen as those most likely to lead to decreased egg production or to a maintenance of production on a level. I have not worked out many of the possible assumptions, but as long as only females belonging to classes 1 or 2 are used in the matings, males of higher classes, *i. e.*, classes 1, 2, etc., are thrown by those of the lower classes in the same manner as pointed out for classes 5, 6 and 9, so that eventually males of the higher classes come to exist in definite ratios.

If class 2 females ($L_1 L_1 L_2 l_2$) only are bred, somewhat different results are secured. If males belonging to classes 1, 2, 3, 4, 7 and 8 are used in equal proportions at the start (classes 5, 6, 9 being omitted for reasons stated above) then in the seventh generation nearly 98 per cent. of the males will belong to class 1, the other 2 per cent. belong to classes 2 and 3, and these will diminish one half in each succeeding generation. The final result, then, should be a race of high producers.

Since it has been shown that when class 1 females are used exclusively in the matings, that eventually a stable flock of birds is produced in which male classes 1, 3 and 7 exist in a definite ratio, and since if class 2 females are bred exclusively a flock consisting solely of high producers will result in time, and since class 1 females mated to males of classes 1, 3 and 7 throw 25 per cent. class 2 females, 50 per cent. class 1 females and 25 per cent. mediocre producers, it would seem inevitable that eventu-

ally a homozygous race of high producers should result from the continued breeding of the sons of high producers to high producers.

Since Pearl has repeatedly stated that his results apply to annual production as well as winter production, it might be supposed that selection during the Gowell régime should have been effective. Pearl has interpreted the results as due to Gowell's failure to use the progeny test. Two other possibilities, however, suggest themselves. Under the Gowell régime the males came from 200-egg hens, but all hens laying over 150 eggs were used as breeders. Possibly the latter value is too low. The annual production corresponding to the 30-egg division-point for winter production has not been stated, as far as I know, but since the mean annual value of a flock of high producers is stated by Pearl to be about 166 eggs, 150 eggs would seem sufficiently high to exclude mediocre producers. The possibility, then, that this value is too low may be disregarded.

The second possibility relates to the number of males used. In an actual experiment only a relatively few males are used each year. The scale at which the Gowell experiments were carried on was hardly sufficient to equal more than one or two years' work on the scale that would be required to get the males in something like the proportions expected, and hence little or no change in egg production could be expected in the period during which the mass selection experiments were carried on.

While, theoretically, mass selection should be effective under the conditions stated, it would require large numbers in order to be uniformly successful. The progeny test, on the other hand, produces results quickly and definitely.

SHORTER ARTICLES AND DISCUSSION

ON SELECTIVE PARTIAL STERILITY AS AN EXPLANATION OF THE BEHAVIOR OF THE DOUBLE-THROWING STOCK AND THE PETUNIA

My attention has only just been drawn to the paper by Howard B. Frost[1] which appeared in the issue of the AMERICAN NATURALIST for October, 1915, under the title of "The Inheritance of Doubleness in *Matthiola* and *Petunia*." In this paper, which is a preliminary communication, the writer states that from a consideration of the data contained in the accounts which I have published of my experiments on the cross-breeding of pure single and double-throwing strains of stocks (*Matthiola*), he has been led to form a view differing from that which I have put forward as to the interpretation to be placed upon these results. Accepting the essential points requiring explanation to be as I have stated them, he discusses the scheme which I have suggested as underlying and accounting for the facts observed, and also the explanation of these facts proposed by Goldschmidt.[2] Though conceding that the factorial scheme which I have formulated[3] would give the results observed, he rejects it on the ground that it is unnecessarily complex, and claims that his own interpretation, which he then gives, is both simpler and supported by definite evidence. Though it is evident that a final decision on the points raised must await further investigation, we can in the meantime examine in the light of our present knowledge the two main grounds upon which Frost claims that his explanation is to be preferred, viz., (1) its greater simplicity and (2) the existence of definite evidence in its favor.

For the purposes of this comparison we need take into account only the three following outstanding facts:

1. That whereas some single stocks yield *only* singles in each successive generation other strains yield a *mixture* of singles and

[1] As Mr. Frost mentions in a note to his paper that he has received no answer to a letter addressed to me in May, 1914, I may take this opportunity to say that no letter or paper from him has ever reached me, and I can only suppose that his letter in some way unfortunately miscarried.

[2] *Zeitschrift f. induktive Abstammungs- u. Vererbungs-lehre*. Bd. 10, p. 74, 1913.

[3] *J. of Genetics*, Vol. 1, No. 4, 1911, and later *J. Roy. Hort. Soc.*, Vol. XL, Part III, 1915.

doubles in each generation. So far as we know the pure-breeding type and the ever-sporting (double-throwing) type differ in no other respect than this particular character. One can obtain parallel strains of the same color, the same habit, the same form of surface character, the one pure-breeding, the other double-throwing.

2. That double-throwing singles give a small but constant excess of doubles, which we have no reason to doubt is due to a constant excess production among the functional gametes of those carrying the double character.[4] Were this excess merely accidental we should expect to obtain deviations from equality in the direction of deficiency as well as of excess, but such deviations have not been found to occur. The excess of doubles suggests a ratio of 9D : 7S ($56\frac{1}{4}$ per cent. of doubles) or possibly 8·5D : 7·5S ($53\frac{1}{8}$ per cent. of doubles).

3. That the results of cross-breeding are such as to show that in the pure-breeding single all the functional egg cells and male germs carry singleness, whereas in the double-throwing strain all the male germs and rather more than half the egg cells carry doubleness, somewhat less than half the egg cells and none of the male germs carry singleness. For if the cross is made in the form no-d single ♀ × d-single ♂ all the F_1 singles give a mixture of singles and doubles in F_2, whereas in the reciprocal cross only a proportion—generally rather more than half—of the F_1 cross-breds behave in this way, the remainder give only singles in F_2.

To explain the above facts I have suggested:

1. That singleness is due to the presence of two factors (X and Y).
2. That in the present-day true-breeding single these two factors

[4] That is to say this excess is not apparently due to any selective mortality after fertilization or germination. In previous accounts I have not introduced into my statements the qualifying expression "functional" but I do not gather that any misapprehension has arisen in consequence, since I have clearly shown that my scheme was based on the results ensuing from fertilization—results, that is to say, arising from the nature of those germ cells which did actually function. Until there was any evidence forthcoming of the formation of non-functional germ cells it seemed more misleading to introduce the word than to leave it out. As, however, Frost bases his view entirely on the supposition that some of the germ cells are constitutionally incapable of functioning it becomes advisable for the sake of clearness to express this limitation, previously inferred but not stated, without, however, prejudging thereby the question of the production of non-functional gametes.

occur linked together ($\widehat{XY}$), *i. e.*, they exhibit the kind of relation which is now generally assumed as the interpretation of a widespread class of phenomena.

3. That in the ever-sporting single this coupling or linkage is only partial so that X and Y can occur dissociated.
4. That in this type of single these two factors are distributed differently to the functional male and female germ cells. The male germ cells are unable to carry either factor. The female germ cells may contain both, or one or other, or neither, the four different combinations occurring in the ratio 7:1:1:7 or possibly 15:1:1:15.

These suppositions provide us with a working hypothesis which covers the facts detailed above and enables us to form a conception of how it comes about that the two classes of singles behave as they do.

If 1 and 2 are true, and they postulate nothing beyond what is held to be the probable explanation in the case of other characters studied by other observers, we must suppose that 3 is also. Since were this not so, the offspring of an ever-sporting single having X and Y linked together in any of its functional germ cells, would some of them behave like F_1 cross-breds from the cross no-d single ♀ × d-single ♂ and would yield only a small proportion of doubles, whereas observation has shown that *all* the offspring of ever-sporting singles give an excess of doubles like the ever-sporting parents. Finally as to 4, the supposition of a sex-limited distribution of factors finds also a parallel in other cases: the special features in the present instance are that a sex-limited distribution of factors should occur in an hermaphrodite organism, and that on the male side it should be complete.

So much for the main points of my scheme which are acknowledged by Frost and clearly set forth by him. I now turn to his criticisms.

1. His first point is that I give no reason *why* singleness rather than doubleness is eliminated on the male side, or why this elimination is *uniform*. This is true. But do we know the reason "why" in any case where factors are lost or where they show a sex-limited inheritance? Take, for example, such a case as the red-flowered stock in illustration of the first point. We know that the original wild form was purple-flowered, and we suppose that at some point a mutant arose from which a certain factor B capable of turning a red color blue, much as an alkali turns red

litmus blue, which was present in the wild form, was eliminated. But we do not know *why* the factor B happened to be eliminated rather than either of the other two factors C and R which we suppose to produce red-colored sap, and to be present in the wild form as well as the factor B. We none the less regard the conception of the existence of the factors B, C and R as satisfactorily expressing the color relations in the stock so far as we know them. Neither are we able to say *why* at some point in the course of evolution a mutant arose, which, although inheriting the factors (or factor) governing singleness, produced some functional germs in which these factors were absent. With regard to his further point, viz., that I offer no explanation of the fact that this elimination is uniform (by which I understand him to mean complete) on the male side in the double-throwing single, seeing that it is partial only on the female side, I may point out that neither does he. Indeed it appears to me to be more difficult to explain the facts regarding the egg cells on his view (see below) than to reconcile the production of a uniform type of male germ with mine. So far indeed as the female germs are concerned he does not attempt to formulate any definite scheme. As it is upon their behavior that the observed excess of double offspring depends, one is constrained to ask why the criticism which he passes on Goldschmidt's scheme, viz., that it "gives at most only an indefinite implied explanation of the deviation of the double-single ratio from equality in the double-throwing races," does not equally apply to his own hypothesis. Stated briefly and apart from certain alternative suggestions put forward tentatively in the absence of any positive evidence, Frost's theory appears to amount to this: that single-carrying as well as double-carrying pollen is formed by the ever-sporting single, but that *all* the single-carrying grains contain some other factor which in some way prevents them from functioning. He finds it further necessary to suppose that a similar lethal process occurs in some of the ovules, but that for some (unexplained) reason *some only* of the single-carrying egg cells are rendered functionless, the others are always capable of fertilization.[5] With regard to his question why it is singleness which is eliminated on the male side and not doubleness, it seems unnecessary to point out that as the double-throwing single has arisen from a pure-breeding single,

[5] I conclude that Frost would accept this as a general statement of his position, but he makes no explicit statement regarding the ovules.

if the mutation is one of loss the character lost must be singleness.

But a more important line of argument is that dealing with the question as to *when* the elimination of singleness takes place. At what stage in the sequence of cell divisions occurring in the direct line between the fertilization of the egg cell at the beginning of the cycle and the formation of the male sperm by the factors, and is the disappearance of the single character from the male germs illusory? In other words, is it the case that both single- and double-carrying pollen grains are formed, but that those carrying singleness always fail to achieve their purpose—fertilization? This brings us to a consideration of the question whether, if elimination occurs, it takes place at some point in the series of cell-divisions which culminate in the formation of the pollen mother cells, or at the reduction division, or at one of the succeeding divisions which give rise successively to the complete sporetetrad, the vegetative and generative cells of the pollen grain, and the twin sperms formed from the generative cell. The first alternative Frost dismisses on the ground that we have no decisive evidence in support of somatic segregation and "an overwhelming convergence of probabilities against it." That we have as yet no actual evidence of such segregation in stocks themselves is true but in the light of known facts in regard to bud variation it seems difficult to escape from the view that somatic segregation not only might, but does sometimes occur, and that this possibility is not excluded in the present case.

It appears to be rather generally assumed, though perhaps without sufficient reason, that the segregation of all allelomorphs must necessarily occur simultaneously. Those who hold that the evidence justifies the belief that segregation takes place at the reduction division are naturally committed to the view of simultaneous separation of all allelomorphs. But it must be acknowledged for the reasons given above that there is a certain difficulty in the way of accepting the view that the reduction division of the germ cells constitutes the sole sorting mechanism for the allelomorphs. May it not be, even if the majority of the allelomorphs segregate as a rule at some particular point, that occasionally or even regularly, segregation of one or another pair of allelomorphs may occur prematurely or again may be postponed? All that we certainly know is that by the time the gametes are formed the sorting has been completed. As regards the possibility of elimination at a later stage than the formation

of the pollen mother cells Frost states that, although he has looked for indications suggestive of degeneration among the spor tetrads, he has seen no signs of it at any stage. I may say that the appearance of the pollen in ever-sporting as in true-breeding singles is that it is all consistently good, whereas, any supposition attributing the behavior of the ever-sporting single to degeneration in the grains must needs assume that about half of them are consistently bad. In the absence of any histological evidence in support of elimination of factors *through degeneration of the pollen grains themselves* Frost does not advance it. Adopting the view (as I gather) that segregation must occur at the reduction division, he does not either consider the possibility of elimination at a later point, though the removal from the cell genealogical tree of one daughter cell in each of two successive divisions of the pollen grain leading to the formation of the twin sperms affords precisely the kind of evidence of which he is in search. There remains the alternative view which Frost puts forward, viz., that both single-carrying and double-carrying pollen is produced, but the single-carrying grains never achieve fertilization. In order to get over the difficulty that the male germs of the true-breeding single are undoubtedly single-carrying but are yet able to effect fertilization Frost is compelled to make one of the following alternative assumptions—and here we come to the main point in his scheme:

1. That the pure-breeding single being regarded as SS in constitution, the double-throwing single must not only be supposed to have lost one S factor, but the remaining original S factor must have become altered, or a new lethal factor completely linked with it must have been produced in the double-throwing mutant "by which the presence of S becomes incompatible with pollen formation." In other words, the two processes which Frost postulates as leading to the appearance of the double-throwing single, require (1) the loss of one S factor, and (2) either the production of a new factor producing singleness which we may indicate by S_1 or the complete coupling of the original remaining S factor with some new factor. That is to say, he also finds it necessary on either view to suppose the existence of at least two factors, S and S_1 and in the second alternative to assume further that the two are completely linked. It seems difficult to see wherein this scheme shows greater simplicity than the one I suggest, which is that singleness is due

to two factors which are completely linked in the true-breeding single type and dissociated in the double-throwing single.

2. As an alternative hypothesis to the above Frost suggests that the pure-breeding single from which the double-throwing single originated may not have been the same kind of single as those which one meets with to-day, but that it differed from them in one or other of the two ways suggested under 1. But again is this a simpler explanation?

Simplicity, however, though not to be disregarded, is not necessarily the final test. Let us consider Frost's second argument that his view can be supported by definite evidence. Failing to find any positive histological evidence that can be taken as indicating the required process of factor elimination, Frost suggests that the case of the ever-sporting single is to be considered as that of a *hybrid showing selective sterility.* To quote his own words:

> Selective partial sterility seems to be rather a common phenomenon, and it very probably occurs here.

Functional sterility is to be supposed in the case of all pollen grains carrying the factor (or factors) essential to singleness, *i. e.*, according to Frost's scheme, either S_1—a modified form of S, or S with a lethal factor linked to it. In other words, half (we are to presume) of the pollen, though apparently good, is supposed to be incapable of fertilizing the ovules. He further adds that if we also assume "a slight tendency to selective elimination of S-carrying eggs"—a somewhat vague supposition to account for a very definite fact—or if these egg cells were less often fertilized than those which are s in constitution, or if there were selective elimination of the embryos (Ss) that would produce singles, we should have "a simple and direct" explanation of the constant excess of doubles. It is in fact suggested that the postulated inability of the single-carrying pollen to fertilize the ovules may be due to want of vigor. As bearing on this point he calls attention to the greater vigor of the double as compared with the ever-sporting single, as shown in the vegetative habit of the growing plant, and in the greater viability of the double-carrying embryos (seed). This greater vegetative vigor of the ss over the Ss zygote Frost contends may possibly be the outcome of a similar difference between the s and S gametes. But if this were so, if the lesser vigor of the ever-sporting single is due to the presence of *one* S fac-

tor, surely we should expect that the true-breeding single, which is SS in constitution, would be less vigorous still. It seems somewhat gratuitous to suppose that the character singleness is sometimes due to a factor S associated with greater vigor and sometimes to a factor S_1 associated with diminished vigor; or, to put it another way, to assume that if it is the lack of one dose of the factor for singleness in the ever-sporting plant which makes it less vigorous, that the lack of the double dose in the double plant leads to the opposite result of greater vigor. Is it not almost a certainty that the greater vigor of the double-flowering plant is due to the fact that the energy of the individual is not exhausted in the formation of the reproductive cells, but is expended in producing a more vigorous vegetative growth? And hence that a check to vegetative growth, similar in cause and in degree, is operative alike in the pure-breeding and the double-throwing single? To obtain strict proof that this is so is difficult since it might always be argued that the particular pure-breeding single strain used as a control was not precisely identical in all other respects with the double-throwing strain with which it was being compared. It can, however, be stated that in some commercial material supplied as double-throwing, but which proved to be a mixture of pure-breeding and ever-sporting singles otherwise apparently identical, no indication was observed of any difference in vigor between the two kinds of singles. The second argument which Frost urges in support of his hypothesis of differential sterility is the fact that the seeds (embryos) which produce doubles have on the whole rather greater viability than their sister seeds which give singles. But this second argument, depending for point on the same entirely unsupported assumption as that derived from vegetative habit, is open to the same objection. I have never observed that the seed-producing true-breeding singles showed any superiority as regards viability over that yielding the ever-sporting singles, but rather, as with vegetative habit, that the distinction is to be drawn between any kind of single-producing seed on the one hand, and double-producing seed on the other. I have sown a large quantity of apparently excellent seed of a pure-breeding single after the lapse of some years and failed to get any germination, just as I have failed sometimes to get any singles from old double-throwing seed although a few doubles were obtained. Lastly, Frost brings forward the somewhat out-of-date view that the percentage of

doubles is increased by starvation treatment. Though this treatment is still practised by German growers, it survives from a time when it was not yet appreciated that the capacity of the individual to become a single or a double depended upon its inherited constitution and not upon the effects of environment.[6] It may be pointed out that the French growers have been in the habit of pursuing precisely the opposite method of treatment with the same object in view. The inhibition of flowering which Frost has observed to be more marked in the singles than in the doubles in the case of one variety when subjected to a high temperature does not seem to me to bear on the question at issue,[7] which is whether there is any direct evidence of the selective sterility of ovules and pollen in the ever-sporting as compared with the true-breeding single. For I gather that Frost is not prepared to maintain that sterility occurs regularly also in the pure-breeding single as well as in the ever-sporting single. It is further to be noted that besides assuming the definite sterility of all the S_1 pollen in an individual of S_1s constitution presumably producing equal numbers of S_1 and s grains, Frost has to have recourse to the vague assumption that there is only a *slight tendency* to selective elimination of the S_1 *egg cells* out of a total composed presumably of equal numbers of S_1 and s. One is fain to ask on what grounds it is possible to uphold the view that the same factor can destroy the functional activity of every pollen grain carrying it, but is only able to affect some of the egg cells in which it is borne? In this connection it may be mentioned that it is no uncommon circumstance even when self-fertilization is left to nature to obtain pods both in pure-breeding and double-throwing singles where every ovule has been fertilized, and this can always practically be ensured where fertilization by hand is carried out in good weather. Though he instances no examples I gather from his previous reference to Belling's work that he has in view such cases as that of *Stizolobium deeringianum* (the Florida Velvet "bean") and other species investigated by that observer,[8] *Nicotiana* on which recent experiments in this connection have been

[6] The real advantage of the German method of treatment is that the seed harvested is all well ripened.

[7] On this subject of the inhibition of flowering, however, Frost promises further information.

[8] *Zeitschr. f. ind. Abst. u. Vererbungslehre*, Band XII, 1914.

made by East,[9] and *Œnothera* studied by Geerts.[10] But in these cases we are dealing with species hybrids, with plants in which partial sterility is a demonstrable fact and due to a demonstrable cause. In the case of *Nicotiana* the parent plants were both (one certainly, the other in all probability) self-sterile and thus the cause of self-sterility in the cross-bred offspring is explained. In *Stizolobium* Belling found, as Geerts had previously noticed in *Œnothera,* that in the hybrids a certain proportion of both ovules and pollen grains were shriveled and malformed. Here again the cause of the sterility is plain. If, as Frost suggests, the ever-sporting single stock should be regarded as a hybrid, it differs completely from the cases referred to above, for in this case there is no obvious sign or cause of sterility either in the plant itself or in the pure-breeding single from which we suppose it to have arisen. It becomes then a question whether to attach weight to the argument from analogy in a case where it goes against all the evidence available.

From the considerations here reviewed I am led to sum up the position as follows:

Evidence is wholly lacking in the stock itself in support of Frost's hypothesis that the behavior of the ever-sporting (double-throwing) single is to be accounted for as the result of the selective sterility of ovules and pollen. Not only so, but it may be claimed that the facts on which he relies to support his argument can equally well be adduced in favor of the opposite point of view. The selective elimination of embryos or the more frequent fertilization of egg cells of s as compared with those of S (or S_1) constitution seem both untenable in view of the fact that the usual excess of doubles is obtained in cases where every ovule is fertilized and every resulting seed germinates. In the formal scheme which I have put forward we have a working hypothesis which enables us to correlate the present known facts. Does Frost's hypothesis give us more than this? Are we not, in the end, still left debating whether his various speculations unsupported by facts really carry us further, and whether they can justly lay claim to the merit of greater simplicity?

There is, however, one further point of interest bearing on this discussion to which I would call attention here. According to the scheme which I have suggested the constitution and gametogenesis of the two kinds of single can be represented thus:

[9] The American Naturalist, Vol. XLIX, No. 578, 1915.

[10] *Récueil des Trav. Bot. Neerl.,* Vol. 5, 1909.

Ever-sporting Single XYxy		Pure-breeding Single (as it is Found in Ordinary Commercial Material) $\widehat{XY}\widehat{XY}$	
Gametes		Gametes	
Ovules	Pollen	Ovules	Pollen
7 XY or 15 Xy	all xy	all $\widehat{XY}$	all $\widehat{XY}$
1 Xy			
1 xY			
7 xy or 15 xy			

the presence of X and Y in the zygote always producing singleness, whether the factors are coupled or not.

Now on this supposition the mating d-single ♀ × no-d single ♂ will give some plants of the constitution XY$\widehat{XY}$. These when self-fertilized will presumably give some pure-breeding F_2 plants of XYXY composition. If these pure-breeding singles in which X and Y are not coupled are crossed back with the pollen of an ever-sporting single we shall again have a plant with the constitution XYxy. In this way we may hope perhaps to synthesize the ever-sporting form. This experiment is already in progress. For this purpose the ever-sporting strain known as sulphur-white is particularly well suited if used as the female parent in a mating with a pure-breeding cream. As the ovules of the sulphur-white appear to be of the four kinds, XYW, XyW, xYw, xyw, we know that *only* F_1 single whites will serve our purpose. We also know that we can disregard among the F_2 families derived from the self-fertilization of F_1 whites those which contain doubles, and proceed to cross individuals in the pure-single families with pollen of an ever-sporting type. By the choice of a sulphur-white as the female parent in the first cross we are saved much trouble in identifying those F_1 plants which contain XY unlinked. In this way we may hope to obtain further light on the different condition of the factors for singleness in the pure-breeding and ever-sporting single, respectively.

After dealing with the stock Frost suggests that his hypothesis of selective sterility no doubt also explains the case of *Petunia*, and adds that in any case my view that singleness is here dominant is untenable. He further mentions that both Goldschmidt and Belling[11] hold the view that doubleness and not singleness is

[11] Belling's statement is that "in the *Petunia* doubtless *may* be incompletely dominant as in the greenhouse carnation." THE AMERICAN NATURALIST, Vol. XLIX, No. 578, p. 126, Note 1, 1915.

dominant in this case. In my account[12] of the results obtained with horticultural strains of *Petunia violacea* and *P. nyctaginiflora* and a hybrid double form I suggested that we might account for the following facts:

1. That the singles when self-fertilized or inter-bred gave only singles.
2. That when crossed with the pollen of doubles they gave both singles and doubles in F_1, the singles being in excess though not always in the same ratio.

by supposing either

(*a*) That some factor essential to singleness was absent from all the ovules of the singles and from some of the pollen of the singles, or conversely

(*b*) That some factor was absent from all the pollen of the doubles, but only from some of the ovules of the singles,

(*c*) Also that more than one factor was concerned in determining the single-double character, and

(*d*) That singleness is dominant.

It will be seen that the above suppositions provide only a general basis of explanation; they do not constitute a full solution even of the facts observed. Frost, putting (*c*) on one side on the ground that it concerns only the deviation of the ratio from 50 per cent., argues that both (*a*) and (*b*) are untenable. He regards the facts as indicating that doubleness and not singleness is dominant, and holds the view that if some of the pollen of the doubles be assumed to carry doubleness and some singleness the hypothesis of partial sterility will explain the rest. But though the formulation of (*c*) was intended primarily to provide for the occurrence of more than one ratio, it was essential also to the suppositions (*a*) and (*b*). If (*c*) is negatived then (*a*) undoubtedly becomes impossible, but if (*c*) is true then (*a*) or (*b*) might represent a basic part of the explanation with which some further complication was combined, as to the nature of which, however, the data available afforded no clue. Owing to the complete sterility of the double plant, it was impossible to make the reciprocal cross. The singles employed might be, in fact almost certainly were, of mixed descent. It was realized that at best either scheme offered only a partial solution. Unfortunately the efforts made in the course of the experiments and since, to obtain seeds of the wild species, have only been partially successful. The position

[12] *Journal of Genetics*, Vol. 1, No. 1, 1910.

still is that we are unable to say for certain whether doubles invariably occur when the above-named species are crossed with the pollen of a double. When this evidence is available we may expect it to throw further light on the question as to which character is dominant. At present decisive proof on this point is lacking. Comparison with the other types carries us no further. Singleness has been found to be recessive in carnation, hollyhock and *Meconopsis cambrica;* on the other hand,—it is dominant in wallflower and probably in sweet william.[13] Moreover, these forms differ from *Petunia* in that they give a *uniform* F_1 when single and double are mated together. The case of *Petunia* therefore still remains one of balance of probabilities. In regard to Frost's further suggestion that the facts observed are due to selective sterility I think that this hypothesis may very possibly be correct and certainly has some evidence in its favor. *P. vidacea* is recognized as a self-sterile species and many of the singles which I used proved to be so. A large number of individuals were tested for this character, but further investigation was postponed in the hope of obtaining pure material for comparison. If Frost's hypothesis is confirmed for *Petunia,* and the work of Belling[14] and East[15] points in this direction, it may offer a complete explanation of the facts and render the supposition of a differential distribution of factors to ovules and pollen in this genus unnecessary.

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GAMETOGENESIS IN PLANTS

THE evolutionary origin of the reproductive cells furnishes one of the most fundamental problems connected with genetics, for upon a clear understanding of the subject depends the satisfactory solution of many subsidiary problems relating to animal and plant breeding. The value of hybridization and inbreeding; the meaning of the pure line hypothesis; the principle of cumulability, etc., may here be mentioned. Therefore, whether or not one agrees with the conclusions presented, studies from widely

[13] Saunders (unpublished). The carnation has also been investigated by Norton. (See paper read at the meeting of the Society of Horticulture in Philadelphia in December, 1904. Also *Gard. Chron.*, Jan., 1905.)

[14] *Loc. cit.*

[15] *Loc. cit.*

divergent standpoints which bear upon the question are to be welcomed. It is only through an analysis of the opinions thus advanced that there will develop a perspective which will eventually permit the solution of the problem.

It is in this connection that the conclusions of Professor Coulter as set forth in "The Evolution of Sex in Plants"[1] are of interest, representing as they do the views of one whose attainments in biology have by no means been confined to the field of plant morphology. Presented in a clear and interesting manner so far as the facts are concerned, the volume furnishes a valuable résumé of the subject from the botanical standpoint. It is evident, however, that a certain narrowness must exist in such a presentation, for a problem of this nature demands that plant biology and animal biology supplement one another from the experimental as well as from the morphological and cytological side. Gametogenesis had its beginning not, as Coulter suggests, among organisms far above the most primitive plants, but among unicellular flagellate forms whose representatives partake of the nature of both plants and animals and from which have arisen the various groups of plants in general. Sexuality, once having arisen, may have been partially or even wholly suppressed in various plant groups, but its subsequent reappearance by no means makes it necessary to affirm its polyphyletic origin. Our present knowledge of Mendelian behavior is of interest in connection with such a view.

It will be well to examine some of the more definite conclusions which Coulter has presented. Few of these are original, nevertheless they are of decided value since they are in most cases supported by unique observations bearing directly upon the point of view. It is merely unfortunate that the bibliographic references which would illustrate the development of the ideas are entirely absent, in consequence of which a false impression may be conveyed to many readers.

Early in the volume it is stated that sex in the higher animals has become the only method of reproduction. Logically this view is not to be maintained, as has already been pointed out by LeDantec ('03) as well as by Chamberlain ('05) evidently in ignorance of the conclusions reached by the previous writer. More recently Janet ('12) has considered the subject. If the criterion by which the sporophyte is to be distinguished from the

[1] By John Merle Coulter, head of the department of botany, Univ. of Chicago. Univ. of Chicago Press, December, 1914.

gametophyte rests upon the $2x$ as compared with the x condition of the chromosomes, we find that among animal organisms the asexual phase has actually become the dominant method of reproduction and the sexual phase is represented only by the parasitic cells arising through the reduction division. In accordance with this view one is prepared to accept the spore mother cells of plants as homologues of the cells preceding the reduction division in animals. In a subsequent discussion Coulter states in accordance with the view first advocated that the animal body produces gametes and not spores. When reduction occurs at the time of the first maturation division in animal organisms it is quite clear that the cells thus produced may correspond to spores which in the next division give rise to gametes. It may even be asserted that they are megaspores or microspores dependent on the sex represented. When chromosome reduction is moved forward to the second maturation division, however, it is possible to agree with Coulter, but seemingly more logical to admit that the change is a secondary one and that the first maturation cells may still represent the spore cells.

In accordance with the proposition that spores unite as gametes to form a single cell, evidence should either be presented to show that an identical chromosome composition exists between the actual spores and the so-called spores functioning as gametes or consideration should be given to subsequent reduction division. Otherwise the conclusion scarcely merits the value of an opinion. Furthermore, the argument that the fusion of a sperm and cell among the angiosperms to form a nutritive endosperm justifies the conclusion that pairing and fusing do not represent the essential features of sexuality, can not be considered. This is only one of numerous examples where changes in form or function of parts occur without having any bearing on the actual origin of the part. Even in this case a fusion is represented and may have a value similar to that among gametes.

It is in connection with "A Theory of Sex" that it seems necessary to decidedly differ from Professor Coulter. Here the two main theses are that sexuality has arisen (1) to carry an organism through an unfavorable environment, and (2) to make evolution more rapid by presenting a greater diversity of forms.

The first deduction is based on the proposition that gametes in many plants are produced at the close of the vegetative period. Such a conclusion—*post hoc ergo propter hoc*—does not rest upon a sound basis. With the fulfilment of a function having the

importance of a gamete production, it is quite logical that the cycle of development should close, but to state that the closing of the cycle has brought about the production of the gametes, is quite another thing. The acceptance of this would lead to the inference that gametes arose in fresh or brackish water forms where pronounced seasonal changes took place and not in larger bodies of water like the ocean, the most probable place.

The second deduction is a restatement of a conclusion reached by Weismann ('76) to the effect that amphimixis increases variability with the assumption that variations thus assumed to be produced are inherited in a cumulative manner. The evidence, however, available at present, supports a view directly contrary to this, namely that the gametic condition makes evolution slower by decreasing the diversity of available forms. Mendelian combinations—amphimutations—may occur but the result is a decrease in variability when the parental populations are compared with the F_2 or with a succeeding generation. The amphimutations are transitory and there is no evidence that they present anything actually new in themselves.

Regardless of the opinions here at variance some of which can only be established as sound generalizations through long experimental investigation, the summary of gametogenesis by Professor Coulter will be read with profit and pleasure by those interested in problems of evolution as well as by those particularly concerned with plant morphology and development.

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NOTES AND LITERATURE

LIFE HISTORIES IN THE RED ALGÆ

THE past decade has given us a number of excellent life history studies in the *Rhodophyceæ* which have been of material assistance in clarifying a very difficult field in plant morphology. The more important of these contributions will form the subject of this review.

Yamanouchi[1] in 1906 published an account of the life history of *Polysiphonia violacea* based on cytological investigations of all of its significant phases. He arrived at the following chief conclusions: (1) Carpospores have 40 chromosomes and tetraspores 20, *i. e.*, half of this number. (2) The sexual plants in their vegetative mitoses showed 20 chromosomes and therefore were believed to arise from tetraspores. (3) The asexual or tetrasporic plants showed 40 chromosomes throughout their vegetative history and consequently could be assumed to come from carpospores. (4) The production on the asexual plants of tetraspores is the result of a reduction division since the first mitosis in the tetrasporangium is clearly heterotypic as shown by the characteristic pairing of the 40 chromosomes and the separation of the members of each pair; thus each tetraspore comes to have 20 chromosomes and is prepared to develop a sexual plant. (5) It was therefore clear that the formation of tetraspores determines the end of a sporophytic generation in the manner characteristic of plants. (6) The gamete nuclei of the sperms and carpogonia take the 20 chromosomes of the sexual plants and fertilization gives the zygote nucleus with 40 chromosomes. (7) Descendants from the zygote nucleus enter the carpospores which consequently have 40 chromosomes and on germination would give tetrasporic plants; certain complicated cell fusions during the development of the cystocarp are purely cytoplasmic in character and evidently nutritional in function. (8) "There is thus an alternation of a sexual plant (gametophyte) with a tetrasporic plant (sporophyte) in the life history of *Polysiphonia*, the cystocarp being included as an early part of the sporophytic phase."

Yamanouchi's contribution on *Polysiphonia* has in great meas-

[1] Yamanouchi, S., "The Life History of *Polysiphonia violacea*," *Bot. Gaz.*, XLII, 401–449, 1906.

ure been the inspiration and largely furnished the basis for the comparative studies that shortly followed. Lewis[2] in 1909 presented an investigation of *Griffithsia Bornetiana* which is in essential agreement with the conclusions of Yamanouchi that an alternation of sexual and tetrasporic plants occurs. From the zygote nucleus, containing 14 chromosomes, are derived the nuclei of the carpospores. The tetrasporic plants have 14 chromosomes and were assumed to come from carpospores. The first mitosis in the tetrasporangium is a reduction division so that 7 chromosomes enter the tetraspores and these were believed to produce sexual plants. Lewis, however, held that the sporophyte generation was represented by the sporogenous cells of the cystocarp and considered the tetrasporic plant to be a phase of an homologous alternation of generations even though it was clear that the tetrasporic plant led up to the critical period of chromosome reduction in the tetrasporangium. From a study of *Griffithsia corallina* Kylin[3] questions the accuracy of the details of nuclear structure and mitoses as given by Lewis and also his count of the chromosomes which in *G. corallina* appear to be as high as in *Polysiphonia.* Kylin describes and figures the reduction phenomena in the tetrasporangium of *Griffithsia* in substantial agreement with Yamanouchi and in an earlier paper gives a similar account for *Rhodomela virgata.*[4]

Extremely interesting are the results of experimental cultures made by Lewis[5] through which fruiting plants have been grown from sporelings established in the laboratory on oyster shells that were then favorably placed in the sea. From carpospores of *Polysiphonia violacea* 6 tetrasporic plants were developed and 23 sterile. Tetraspores of *Griffithsia Bornetiana* produced a total of 60 sexual plants (32 male and 28 female) and 15 sterile. Tetraspores of *Dasya elegans* gave 149 sexual plants (143 male and 6 female) and 139 sterile, the apparently large proportion of male plants in the culture of this species probably being due to slower maturing of the females. The sterile plants of these cultures were

[2] Lewis, I. F., "The Life History of *Griffithsia Bornetiana*," *Ann. of Bot.*, XXIII, 639–690, 1909.

[3] Kylin, H., "Die Entwicklungsgeschichte von *Griffithsia corallina* (Lightf.) Ag.," *Zeitsch. f. Bot.*, VIII, 97–123, 1916.

[4] Kylin, H., "Studien über die Entwicklungsgeschichte von *Rhodomela virgata* Kjellm.," *Svensk. Bot. Tidskr.*, VIII, 1914.

[5] Lewis, I. F., "Alternation of Generations in Certain *Florideæ*," *Bot. Gaz.*, LIII, 236–242, 1912.

of course individuals which at the time of the examination had not yet developed fruiting organs. More extended studies of Lewis[6] have established the seasonal habits at Woods Hole, Massachusetts, of the above species of *Polysiphonia, Griffithsia* and *Dasya.* Tetraspores and carpospores were germinated on shells that were fastened to piles and left over winter. In June tetrasporic plants of carposporic origin were abundant, which, releasing their tetraspores in July, produced a crop of sexual plants that matured their carpospores in August or early September. The small plants from the carpospores winter over and produce the tetrasporic plants of the first summer generation. Thus the sexual generation is conspicuous in the late summer while tetrasporic plants surviving the winter are characteristic of the spring. Belated growth of tetrasporic plants may result in their fructification late in summer, so that a few small carposporic plants also winter with the tetrasporic but they are relatively scarce. The seasonal history is then in the main characteristic; tetrasporic plants appear in the spring and through their spores produce a summer crop of sexual plants, from the carpospores of which a generation of small tetrasporic plants in favorable situations carries the species through the winter. This experimental work thus supports at all points the conclusions of Yamanouchi based on cytological studies.

The most important cytological work on life histories in the red algæ since the paper of Yamanouchi on *Polysiphonia* is that of Svedelius presented in studies on *Martensia, Delesseria, Nitophyllum* and *Scinaia.* Part of this work, described in the next paragraph, concerns the development of tetraspores in multinucleate tetrasporangia (*Martensia* and *Nitophyllum*). All of it supports to the fullest degree Yamanouchi's theory of alternation of generations in such species as have tetrasporic plants in their life histories. The work on *Scinaia* presents a most interesting hypothesis for the life histories of such red algæ as do not develop tetraspores.

The life history of *Delesseria sanguinea* is discussed by Svedelius[7] in three papers embodying cytological work of a high

[6] Lewis, I. F., "The Seasonal Life Cycle of Some Red Algæ at Woods Hole," *Science*, XXXIX, 253, 1914.

[7] Svedelius, N., "Ueber den Generationswechsel bei *Delesseria sanguinea*," *Svensk. Bot. Tidskr.*, V, 260–324, 1911. "Ueber die Spermatienbildung bei *Delesseria sanguinea*," *ibid.*, VI, 239–265, 1912. "Ueber die Zystokarpienbildung bei *Delesseria sanguinea*," *ibid.*, VIII, 1–32, 1914.

order. Tetrasporic plants have 40 chromosomes in their nuclei. This number is reduced by the first nuclear division in the tetrasporangium. During synapsis the 40 chromosomes become grouped to form 20 pairs, which become exceptionally clear in the stage of diakinesis. The first or heterotypic mitosis separates the members of the pairs, thereby halving the number and giving after the second mitosis 20 chromosomes to each of the tetraspores. Vegetative mitoses in the male and female plants show uniformly 20 chromosomes and it must be assumed that these sexual plants develop from tetraspores. The chromosomes are organized in the prophases of mitosis directly from a chromatic network and without the interpolation of a spirem stage. The spermatangia are cut off in pairs to the right and left of a mother cell and in each spermatangium a uninucleate sperm is organized (20 chromosomes) which on escaping leaves behind an empty cyst. The young carpogonium is uninucleate but an early division differentiates the female gamete nucleus (20 chromosomes) which remains in the carpogonium, and a trichogyne nucleus that shortly breaks down. The carpogonium terminates a 4-celled carpogonial branch borne by a cell ("tragzelle") from which develops also an auxiliary cell and certain sterile cells. The sterile cells enlarge greatly after the fertilization of the carpogonium and then break down, forming a mass of slime which apparently serves to give space and protection for the development of the gonimoblasts. In some way not clearly understood the zygote nucleus of the fertilized carpogonium enters the auxiliary cell and from this cell as a starting point the gonimoblasts arise as a dense growth of short filaments. The cells of the gonimoblasts contain nuclei derived from that of the zygote and consequently have 40 chromosomes which are passed on to the carpospores developed in rows. From the carpospores must come the tetrasporic plants with their 40 chromosomes. We have therefore in *Delesseria* an antithetic alternation of generations exactly parallel with that of *Polysiphonia*, a diploid phase including the gonimoblasts and the tetrasporic plants alternating with a haploid phase represented by the sexual plants.

Svedelius[8] opened a new vista in cytological studies on the red algæ with his discovery of multinucleate tetraspore mother cells in *Martensia*, one of the *Delesseriaceæ*. The young cell has

[8] Svedelius, N., "Ueber den Bau und die Entwicklung der Florideengattung *Martensia*," *Kungl. Svensk. Vet.-akad. Handl.*, XLIII, No. 7, 1908.

like its neighbors several nuclei which by division increase in number as the cell enlarges until about 50 are present. Then a general nuclear degeneration sets in coincident with an increase in the amount of cytoplasm and only one nucleus in the center of the plasma mass survives to give rise to the 4 nuclei that enter the tetraspores. A similar situation was found by Svedelius[9] in *Nitophyllum punctatum,* a related form of the same family, which is described in greater cytological detail. Typical mitoses in the young tetraspore mother cell give it a dozen or more nuclei all with about 40 chromosomes, the diploid number for the species. Many of the nuclei shortly begin to show signs of degeneration by the disappearance of the chromatin so that only the nucleolus remains to take the stain, and there is also a shrinkage of the nuclear membrane. The degeneration is not simultaneous, a few nuclei increase in size and give indications of preparation for the heterotypic mitosis as shown by clear stages of diakinesis. However only one nucleus carries the history of reduction further and thus becomes the surviving nucleus of the tetraspore mother cell, a nucleus very much larger than the degenerating structures that lie about it in the cytoplasm. At diakinesis pairs of chromosomes are clearly shown, about 20 in number. The members of the pairs are separated by the first mitosis which is therefore heterotypic and a reduction division as in *Polysiphonia, Griffithsia* and *Delesseria.* The second mitosis, homotypic, gives the four nuclei of the tetraspores, each with about 20 chromosomes. It is very interesting to note that the red algæ present illustrations of nuclear degeneration at a period of reproduction when it may be desirable to conserve the cytoplasm of a cell for a limited number of reproductive elements. This nuclear degeneration appears to be strictly analogous from a physiological point of view to that exhibited in the oogonia of *Vaucheria, Saprolegnia* and *Albugo,* in the sporangium of *Derbesia,* and in the oogonia of certain forms of the *Fucaceæ.*

With the cytological and experimental evidence in complete accord and so strongly in favor of the theory of an antithetic alternation of generations in those red algæ which have tetrasporic plants certain observations which at first thought appear to offer exceptions to this theory naturally take on a high degree of importance. The literature records a number of species which

[9] Svedelius, N., "Ueber die Tetradenteilung in den vielkernigen Tetrasporangiumanlage bei *Nitophyllum punctatum,*" *Ber. deut. bot. Gesell.,* XXXII, 48–57, 1914.

have been reported to bear tetrasporangia on sexual plants, facts which would be significant if it were established that such tetrasporangia were the seat of reduction divisions. Three of these species have been studied cytologically and there is good evidence that the reduction divisions are not present and that tetraspores are either not fully matured or that the "tetrasporangium" develops a monospore. Yamanouchi and Lewis in their studies found occasional sexual plants bearing what seemed to be tetrasporangia but in these cells the nucleus remained undivided in *Polysiphonia* or produced several nuclei in *Griffithsia* while cleavage furrows proceeded only a short distance into the cytoplasm. Svedelius[10] has reported on a cystocarpic plant of *Nitophyllum punctatum* bearing tetrasporangium-like structures. These cells were found around points in the thallus where procarps had been formed and only reached their fullest development when the procarps remained unfertilized. Their position on the thallus therefore indicated a close correlation with the nutritional physiology of the plant. These cells in their early history follow exactly the course of normal tetrasporangia in this species; there is a multiplication of nuclei and then a degeneration of all but one which takes its position in the center of the cell. The surviving nucleus does not divide but the entire protoplast slips out of the thallus as a uninucleate monospore. Since the cystocarpic plant was haploid a reduction division in this tetrasporangium-like cell would have been most irregular; it does not take place and the monospore in its chromosome count has the same value as a tetraspore. *Agardhiella* (*Rhabdonia*) *tenera* presents another problem brought forward by observations of Osterhout. Tetraspores of this plant sometimes germinate while still imbedded in the tissues of the parent with the further peculiarity that the tetrad group behaves as a unit so that all four cells enter into the formation of a sporeling. These epiphytic sporelings commonly become sexual plants, as would be expected from the germination of tetraspores. Osterhout, however, reports that occasional tetrasporic plants are developed which would be irregular unless it were found that such plants came from tetrasporangia in which reduction divisions had been suppressed so that such tetrasporangia, behaving like monospores, give rise to diploid plants (tetrasporic) similar to the generations on which

[10] Svedelius, N., "Ueber Sporen an Geschlechtspflanzen von *Nitophyllum punctatum*; ein Beitrag zur Frage des Generationswechsels der Florideen," *Ber. deut. bot. Gesell.*, XXXII, 106–116, 1914.

they were borne. It is probable that other red algæ will exhibit peculiarities of life history related to apospory, as seems probable in this case of *Agardhiella*.

There is enough evidence before us in these three studies by Yamanouchi, Lewis and Svedelius to demand the utmost caution in the consideration of other cases of "tetraspores" upon sexual plants which have been brought forward by critics of the theory of alternation of generations as applied to the red algæ, cases which have not as yet been subjected to the tests of cytological research. One of the most interesting of these is *Platoma Bairdii* (Farl.) Kuckuck[11] which in Helgoland apparently produces no antheridia but develops cystocarps parthenogenetically. On these cystocarpic plants are also found tetraspores indicating that the plants are diploid in character which if true would account for their apogamous behavior. *Platoma Bairdii* therefore appears to be one of the cases in which sexual organs are developed upon a diploid plant and not one in which tetraspores are found on a haploid generation. A cytological study of this form would be a matter of great interest and the only way in which the facts may be determined. Also, there are the problems of the paraspores or polyspores characteristic of a number of species in the *Ceramiaceæ*. These are spores borne numerously in chains (seirospores) or in dense glomerules and are found on tetrasporic plants. Schiller[12] regards them as homologous with tetraspores, and as supporting the view of Oltmanns that the latter are reproductive spores without significance for an alternation of generations, but until we know the cytology of their development an opinion can have little value. Should the paraspores be formed without reduction divisions, as seems probable, they will rank in the same class with monospores and play no part in an alternation of generations. Should there be found evidence of a heterotypic mitosis in the development of these exceptional reproductive cells they might perhaps constitute a modification of the tetrad group characteristic of reduction divisions, but it does not seem likely that this will prove to be the case.

There is left for consideration those red algæ which produce no tetraspores at any phase of their life history. They include a number of well-known types such as *Nemalion*, *Batrachosper-*

[11] Kuckuck, P., "Ueber *Platoma Bairdii* (Farl.) Kck.," *Wiss. Meeresuntersuch. Biol. Anstalt Helgoland.*, V, 187–203, 1912.

[12] Schiller, J., "Ueber Bau, Entwicklung, Keimung und Bedeutung der Parasporen der Ceramiaceen," *Oester. bot. Zeitsch.*, LXIII, 144, 203, 1913.

mum, *Scinaia*, *Helminthora*, etc., and at present constitute the field where research is most needed in connection with the life histories of the red algæ. Apart from asexual monospores the reproduction takes place only through carpospores produced in simple cystocarps. There are in the life histories no groups of tetrad cells to suggest the position of reduction divisions which explains the reasons why our understanding of alternation of generation in the *Rhodophyceæ* has come chiefly through higher forms exhibiting the tetrasporic phase. The first cytological research on a life history among the forms lacking tetraspores was the study by Wolfe[13] of *Nemalion*. This investigation brought out a number of important discoveries: the presence of a nucleus in the trichogyne, the mitosis which gives two sperm nuclei after the fusion with the trichogyne, the proliferation of the cells terminating the sporogenous filaments of the cystocarp to form successive carpospore mother cells, the structure of the chromatophore. With respect to the chromosome count in the life history Wolfe concluded that vegetative mitoses presented about 8 chromosomes and that 16 is probably the number shown by the mitoses in the developing cystocarp. The period of chromosome reduction was placed in the terminal cells of mature sporogenous filaments, one of the daughter nuclei, following a reduction mitosis, passing into a carpospore mother cell. The other reduced daughter nucleus was assumed to become established in the cell from which successive carpospores are produced by proliferation. The cytology of *Nemalion* is admittedly difficult and in the absence of a conspicuous group of four nuclei characteristic of reduction divisions (such as is found in the tetraspore mother cell) it is natural that critics of Wolfe's conclusions should have suggested other possible positions in the life history for the critical period of chromosome reduction. Thus it was suggested that reduction might take place at the germination of the carpospore, but Lewis[14] in a short note reports that the first and later mitoses in the sporelings are vegetative mitoses with about 8 chromosomes. A second possibility, that reduction takes place in the first division of the zygote nucleus in the fertilized carpogonium, is strongly debated by Svedelius in his recent paper on *Scinaia*.

[13] Wolfe, J. J., "Cytological Studies on *Nemalion*," *Ann. of Bot.*, XVIII, 607–630, 1904.

[14] Lewis, I. F., "The Germination of the Spore of *Nemalion multifidum*," *Science*, XXXV, 154, 1912.

Svedelius[15] gives a cytological study of *Scinaia furcellata* which suggests some fundamental readjustments of our conception of the cystocarp in those red algæ which lack the tetrasporic phase in their life histories. Monosporangia and spermatangia occur on the same plants growing out of and becoming cut off from mother cells at the surface of the thallus with evidence that successive crops may be formed from the same mother cells. Both monosporangium and spermatangium have a nucleus with about 10 chromosomes and the similarity of their structure and manner of development indicates that they are homologous organs. The 3-celled carpogonial branch requires a somewhat detailed account since its history presents phases not previously described for the red algæ. The terminal cell becomes the carpogonium and a mitosis gives a nucleus to the trichogyne as has been described for *Batrachospermum, Nemalion, Polysiphonia, Rhodomela, Delesseria* and *Griffithsia*. From the second or hypogenous cell is formed a group of 4 auxiliary cells rich in protoplasmic contents. The third or basal cell of the carpogonial branch develops finally the envelope of the cystocarp. The nuclei of the carpogonial branch including the female gamete nucleus in the carpogonium have 10 chromosomes which is the haploid number of the *Scinaia* plant. After the fertilization of the carpogonium the zygote nucleus, with 20 chromosomes, and consequently diploid, passes into one of the auxiliary cells which have become more or less fused together. In the auxiliary cell the large zygote nucleus prepares for and passes through a heterotypic mitosis with apparently clear evidence of diakinesis shown in the presence of 10 pairs of chromosomes. A second mitosis gives the tetrad of 4 nuclei but only one of these becomes concerned with the development of the gonimoblasts, passing back into the carpogonium from which the gonimoblasts arise. The other 3 homologous nuclei of the tetrad together with the nuclei of the auxiliary cells take no further part in the history of the cystocarp. Nuclei of the gonimoblasts have 10 chromosomes and this number is passed to the carpospores which are formed successively in rows; some of the gonimoblasts remain sterile and develop into long filaments resembling paraphyses.

A most unexpected outlook upon the life histories of the red algæ will be opened if these conclusions of Svedelius on *Scinaia* are confirmed and if studies on *Nemalion, Batrachospermum*, etc.,

[15] Svedelius, N., "Zytologisch-Entwicklungsgeschichtliche Studien über *Scinaia furcellata*," *Nov. Act. Reg. Soc. Scien. Upsal.*, IV, No. 4, 1915.

should also determine the position of chromosome reduction to be at the first mitosis of the zygote nucleus. The cystocarps of these plants would then be interpreted not as a diploid sporophyte generation but as a special haploid phase and the carpospores would have the same value as monospores. There would be no antithetic alternation of generations in such forms of the red algæ. Svedelius proposes the term *haplobiontic* for red algæ with this type of life history to be contrasted with *diplobiontic* forms (*Polysiphonia*, *Rhodomela*, *Griffithsia*, and *Delesseria*) where the gonimoblasts have been shown to be diploid in character and chromosome reduction finds its place in the terasporangia of an asexual generation. The diplobiontic type of life history is naturally conceived by Svedelius to arise from the haplobiontic by a delaying of the reduction divisions so that gonimoblasts carry forward the diploid number of chromosomes to the carpospores. The carpospores being diploid develop an asexual generation and their diploid sporangia becoming the seat of the reduction divisions take the characters of tetrasporangia, a new type of reproductive organ. The occasional suppression of reduction divisions in the tetrasporangium may be expected at times to transform this cell to a monosporangium, as in *Nitophyllum,* showing close analogies between the two structures.

In connection with the conclusions of Svedelius on *Scinaia* it should be remembered that Allen has placed the reduction period in the life history of *Coleochæte* at the first mitosis of its zygote nucleus. If this view is correct the cellular body developed in the oospore of *Coleochæte,* preliminary to the formation of a crop of zoospores, is a special haploid structure and may be compared with the gonimoblastic development in *Scinaia* as interpreted by Svedelius. The haplobiotic red algæ of Svedelius then, if clearly established, would have the same type of life history as *Coleochæte* and bearing in mind the resemblance of the young oogonium of *Coleochæte* to a carpogonium and trichogyne, the resemblance of its antheridia to a cluster of spermatangia and the branched filamentous structure of its thallus, the view that *Coleochæte* represents a type from which the red algæ might have arisen takes on added strength.

It is interesting to note how far removed from the theories of Schmitz and Oltmanns are the more recent interpretations of the life histories in the red algæ. The secondary or double fertilization assumed by Schmitz as an explanation of the union of ooblastema filaments with auxiliary cells was discredited by Olt-

manns's work and has received no support from the cytological studies of Yamanouchi, Lewis, Kylin and Svedelius. Oltmanns's view that tetraspores have no fixed place in ontogeny and are without relation to a sporophyte generation has been overwhelmed by the cytological work of the authors mentioned above. And now Svedelius argues that even the old view that cystocarps represent a sporophytic phase can not be correct for a group of the red algæ which he terms haplobiotic. The situation as it now stands may be summarized as follows: An antithetic alternation of generations may be expected wherever tetrasporic plants are found in a life history and in these forms the gonimoblasts also constitute a phase of the sporophyte. There is no sporophyte in the "haplobiotic" red algæ (*e. g.*, *Scinaia* with *Nemalion*, *Batrachospermum*, etc., not yet studied from this standpoint) and the gonimoblasts of these forms represent a haploid development of the zygote in position upon the plant, carpospores being equivalent to monospores. Fusions with auxiliary cells are merely cytoplasmic in character and associated simply with nutritive functions. The diplobiotic red algæ have come from the haplobiotic, which, carrying forward the reduction divisions through the gonimoblasts and carpospores to a new generation, the tetrasporic plants, have established the reduction divisions in the tetrasporangium. In theory these views are simple and logical. For the antithetic alternation of sexual and tetrasporic plants the evidence is considerable and convincing. What will be the conclusions for the "haplobiotic" types? Will future studies establish their existence?

BRADLEY MOORE DAVIS

UNIVERSITY OF PENNSYLVANIA,
July, 1916

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THE STATUS OF THE MUTATION THEORY, WITH ESPECIAL REFERENCE TO ŒNOTHERA[1]

DR. HARLEY HARRIS BARTLETT

The more or less controversial literature of the mutation theory is so scattered and involved that few except specialists have been able to follow it with any degree of satisfaction. A recent book by Gates, "The Mutation Factor in Evolution, with Particular Reference to *Œnothera,*" will help the general biologist to an understanding of the situation, but even in the short time since it went to press there have been developments of such significance that I am glad of this chance to review the subject.

The crux of the whole controversy is this: Are the discontinuous variations which occur in cultures of Œnothera true mutations, which might appear either in pure lines or in hybrids, or are they segregates from genetically impure lines? The mutationist and his Mendelian critic give diametrically opposite answers to this question. Some geneticists are beginning to feel that there is justification for taking a middle ground, that the mutations are non-Mendelian, but that they are nevertheless a phenomenon of hybridism, and occur only in impure lines.

The most extensive researches upon mutability have been carried out with *Œnothera Lamarckiana.* This

[1] Papers from the Department of Botany of the University of Michigan, No. 150. Read before the American Society of Naturalists at Columbus, Ohio, 30 Dec., 1915. Based in part upon unpublished experiments conducted at the U. S. Bureau of Plant Industry and published with the permission of the Secretary of Agriculture.

plant gives rise to mutations of two main types, those in which the chromosome number differs from that of the parent form, and those in which it does not. The most striking ones belong to the former category; among them we need mention particularly only *Œ. gigas* and *Œ. lata,* which have, respectively, 28 and 15 chromosomes, instead of 14, the normal number in the species. Gates has especially emphasized the fact that in the mutating Œnotheras the pairing of the chromosomes, previous to the reduction division, is very loose, a condition that would favor irregularities in the distribution of the chromosomes to the gametes. By irregular reduction divisions, gametes with a greater or less number of chromosomes than 7 might easily be formed. From these irregular gametes there would be derived, in turn, zygotes with irregular chromosome numbers. Mutationists are now pretty well agreed that the characters of certain mutations are correlated with an unusual complement of chromosomes. If one believes at all that the chromosomes provide the mechanism of Mendelian inheritance, it is hard to escape the conclusion that the cytological studies of Lutz, Gates and others give a firm basis for removing at least part of the mutation phenomena from the domain of Mendelian segregation.

Even Davis, who has been one of the chief opponents of the mutation theory, has admitted that some of the variants from *Œnothera Lamarckiana* and other species are probably due to irregularities in chromosome distribution. He makes the point, to be sure, that the unpaired condition of the chromosomes previous to reduction in *Œ. Lamarckiana* is itself exceptional, and presumably the result of a hybrid constitution. Davis himself has shown that in at least one of the numerous strains of *Œ. grandiflora* which occur at the type locality of that species in Alabama, the chromosomes, in preparation for the reduction division, become associated definitely and closely into ring-shaped pairs. Moreover, this same strain shows a negligibly low pollen and seed sterility. Davis views it as essentially a genetically pure strain, whether judged by

its morphology or by its physiological behavior. In 1912 de Vries and the speaker visited the locality from which Davis's strains came, and found such a confusion of different types growing together that it was impossible to doubt that the entire Œnothera population was hybridized to a greater or less degree. Some of the forms belonged to the series of *Œ. grandiflora,* being large-flowered and open-pollinating, whereas others were small-flowered, self-pollinating types, showing obviously the effects of hybridization with *Œ. Tracyi,* another southern species. It is curious that the particular *Œnothera* to be put forward as probably genetically pure, judged by Davis's cytological criterion, or by Jeffrey's pollen test, should come from a locality where hybridization is so prevalent that one would hardly expect to find among the open-pollinating forms a single unhybridized plant. It will be remembered that Jeffrey has attacked the mutation theory from the point of view that pollen abortion necessarily indicates hybridity. By this criterion the small-flowered practically cleistogamous species which self-pollinate generation after generation must be adjudged highly impure, although the evidence is all to the contrary. There is every indication that pollen abortion is a frequent concomitant of mutation, as well as of hybridization. It seems not unlikely, therefore, that the unpaired condition of the meiotic chromosomes may have a causal relationship with pollen abortion as well as with the production of those types of mutations which have a chromosome number different from that of the parent species.

It is ordinarily supposed that a mutation is determined when the reduction division takes place. This may be the case with the mutations in which there are irregular chromosome numbers. Even in such cases it may be that the germ plasm has undergone at some other point in the life cycle a premutative modification of such a nature as really to predetermine the kinds of mutated gametes which will subsequently appear. A physiological premutation might, for instance, bring about the condition which results directly in loose chromosome pairing, and

indirectly in the formation of several different types of mutated gametes. Many mutations are not concerned with such obvious changes as the shifting of chromosomes, but seem rather to depend upon physico-chemical and chemical alterations of the germ plasm. Obviously some such alterations would result in physiological as well as morphological mutations. Just as certain morphological changes are not advantageous, or even distinctly harmful, so certain physiological changes might be harmful and lead to sterility. Some premutations (and by a premutation I mean the inauguration of an unstable condition in the germ plasm) might be of such a nature that the nutrition of the spore mother cells would be interfered with. These might fail to develop, fail to undergo the reduction division or might give rise to defective daughter cells.

Thus mutation, equally as well as hybridization, may account for sterility. There are several groups of plants in which sterility has apparently come about without the possibility of hybridization. Perhaps Davis's genetically pure *Œnothera grandiflora*, with perfect pollen, provides a case of hybridization without subsequent mutability. Those who assert that germinal instability comes about only by hybridization can bring forward no proof of their assertion. Conversely, the mutationist can not prove that any plant in existence has had an unmixed ancestry. The most that he can do at present is to show that mutation takes place in strains which are genetically pure, and that the purity is of relatively long standing. One can only conclude that Davis's and Jeffrey's suggested cytological and morphological evidences of hybridity, if verified, will merely substitute hybridization for premutation as a cause of germinal instability. They will not in any way afford support to the Mendelian conception of mutation.

Nothing could be more obvious than the paths which are marked out for the student of the *Œnothera* problem by the interesting cytological clue afforded by the unpaired chromosomes of *Œnothera Lamarckiana* and other

species. Some one must find out whether or not the unpaired condition occurs in hybrids whose parents do not show it. Strange as it may seem, after all the discussion of hybridity as a possible cause of mutability, no one has yet shown, or tried to show, that mutability occurs in any hybrid between non-mutable parent species. This would seem to be one of the most crucial experiments that could be performed, and one of the easiest. It would be a very attractive problem to attempt to produce the unpaired chromosome condition by hybridization and then to prove it definitely correlated with the particular types of mutability which are characterized by disturbances of the chromosome mechanism.

Even if it were possible in the time at my disposal to review the evidence that the chromosomes provide the mechanism of inheritance, it would hardly be necessary to do so. The brilliant work of Morgan and his students on the association in *Drosophila* of groups of characters with definite chromosomes is well known to every one. In *Œnothera* the investigations of Gates, Lutz and others have shown a connection between chromosome alterations and the characters of certain mutations so obvious that it can not reasonably be disregarded.

There are still a few geneticists, however, who believe that the chromosome cycle has no fundamental significance in connection with Mendelian phenomena. Heribert-Nilsson would ascribe as much weight to a change from flat to crinkled leaves, to choose an example at random, as he would to a change from the 2x to the 4x chromosome number. Such an attitude is forced upon one who attempts to explain all mutations as Mendelian segregates, as this author does. He has made much of a case, observed by Geerts and Stomps, in which the chromosome number of a hybrid between *Œnothera gigas* and *Œ. Lamarckiana* became reduced from 21 to 14, probably through the agency of an irregular reduction division, without the loss of the *gigas* characters. This case is cited to prove that the characters of this mutation do not depend upon the supernumerary chromosomes. Nothing

is more probable, however, than that the chromosomes are qualitatively different, and that the *gigas* characters depend upon the duplication of some, not all, of the chromosomes. An irregular reduction division might well result in the retention in duplicate of those particular chromosomes upon which the *gigas* characters depend. The very characteristic aspect of *Œnothera lata* has been ascribed to its single supernumerary chromosome. Miss Lutz has shown that many mutations with 15 chromosomes do not have at all the characteristic *lata* appearance, which must therefore be attributed to the duplication of a particular chromosome, rather than of any chromosome.

The production of mutations with irregular chromosome numbers is not confined to *Œnothera Lamarckiana*. Two other species have given rise to mutations with 28 *chromosomes*, and in one case, that of *Œ. stenomeres*, the *gigas* mutation is entirely comparable in its characters with *Œ. gigas* de Vries. Its wood structure has been compared with that of the parent species and has been found to present deviations as great as those which are apparent in the external aspects of the two plants. The differences concern not only the relative size of the elements, but also their shape, and, to a certain extent, their distribution. In typical *Œ. stenomeres* the medullary rays are sometimes 140 cells high, whereas in mutation *gigas* they are typically less than 25 cells high, and as far as we have observed, never over 50. It is very significant indeed that striking structural alterations in the most conservative tissues of a plant may be instituted by a single mutative evolutionary step.

As far as the mutations with modified chromosome numbers are concerned, there is the best of evidence that the processes of mutation and Mendelian segregation are absolutely distinct and independent. The evidence is not only cytological, but also genetical, for no mutations of this class show Mendelian inheritance when crossed with their parent forms. Their significance in evolution is illustrated by many widely separated groups of plants in

which species or genera are set apart from their allies by the possession of a different number of chromosomes. Such variations in chromosome number have been found in many cases among the Rosaceæ, a family noteworthy for the complications which it presents to the systematist. Certain Japanese species of Viola exhibit variations in chromosome number which give a clue to the way in which the numerous forms of this complex genus have evolved. Similar variations occur among the Orchidaceæ, one of the largest families of flowering plants.

There remain to be considered a large number of mutations in which the chromosome complement has not been shown to differ from that of the parent form. Such mutations are frequent in *Œnothera*. De Vries has observed them in the case of *Œ. Lamarckiana* and more recently he and other workers have observed them in other species, belonging to the small-flowered, self-pollinating portion of the genus. In connection with these mutations it will be necessary to consider more in detail the criticisms brought against the mutation theory by Bateson, Davis, Heribert-Nilsson and others.

In the past, most objections to the mutation theory have been based upon the supposition that *Œnothera Lamarckiana* is a hybrid of garden origin. I am forced to admit that I am not satisfied with any evidence thus far offered that this species, in the form familiar in cultivation, is or ever was a wild constituent of our flora. Nevertheless I venture to predict that it will eventually come to light in some obscure locality and that its character as a natural species will be established. Whether it is a natural species or a product of floriculture is of relatively little importance, however, in view of the fact that none of the mutation phenomena are peculiar to it. Several other species are known which are equally mutable and which are now elements of our flora. Moreover, they are small-flowered, self-pollinating forms, and therefore better suited to mutation studies than large-flowered, open-pollinating forms such as *Œ. Lamarckiana*, which in nature must frequently be hybridized.

Before mutation studies had been extended to other species of the genus from *Œ. Lamarckiana,* Davis began a series of experiments with the object of reproducing the latter species as a hybrid of known origin. His first experiments, involving *Œ. grandiflora* as one parent, were unsuccessful in producing a plant that bore more than a superficial resemblance to *Œ. Lamarckiana.* Some of the hybrids showed mutability, but none were obtained which did not show obvious segregation in addition to the mutability. Moreover, the mutations were not shown to have been induced by hybridization, since none of the parent strains were tested for constancy. As de Vries suggested, the mutability was probably an inherited tendency from one or both parents.

Later hybrids, between *Œ. franciscana* and *Œ. biennis,* were much more successful, in that they bore a much closer resemblance to *Œ. Lamarckiana.* The writer saw hybrids last summer in Davis's garden that would surely have been placed by any except the most ultra-critical systematist under *Œ. Lamarckiana.* They are being carried into another generation, and the results will be looked forward to with much interest. A true synthetic *Œ. Lamarckiana* must show mutability, but must otherwise come true from generation to generation. Moreover, it must give twin hybrids in certain crosses with other species. Even if Davis's later hybrids fulfill these conditions, they will not demonstrate the origin of mutability through hybridization, for one of the parents, *Œ. biennis,* has been shown by de Vries and Stomps to be a mutable species, and the other, *Œ. franciscana,* has not been tested. To have much weight, an experiment such as Davis's must show the origin of mutability *de novo* in a hybrid from non-mutable parents.

A more recent phase of the effort to prove *Œ. Lamarckiana* a hybrid dates from the publication, in 1914, of a paper by O. Renner. This author proposed a simple Mendelian hypothesis to account for the twin hybrids and high seed sterility of *Œ. Lamarckiana.* It is well known that in this species about half of the seeds are empty or

do not contain normal embryos. Moreover, when crossed with certain species, the first hybrid generation consists of two types, the twin hybrids of de Vries. Renner assumes that *Œ. Lamarckiana* is heterozygous and that it produces two types of functional gametes. Its progeny under ordinary circumstances would therefore be expected to consist of recessive homozygotes, heterozygotes, and dominant homozygotes in the familiar 1:2:1 ratio. He further assumes, however, that the homozygotes are incapable of developing beyond a young embryonic stage, and that the species is therefore maintained in a heterozygous condition from generation to generation. This simple hypothesis obviously does not account for the mutability of *Œ. Lamarckiana*. It has been amplified with this end in view by Heribert-Nilsson, whose highly involved explanation of mutability from the standpoint of the plural factor hypothesis must receive a brief consideration. For several years this worker has busied himself in an attempt to demonstrate Mendelian inheritance in *Œ. Lamarckiana*. In one case he thought he had found simple monohybrid segregation in crosses between red- and white-nerved races, and announced that the nerve color acted as a simple Mendelian character. It developed later, however, that his ratios were aberrant, and that the progenies entirely lacked a class of plants homozygous with regard to the supposed dominant character. According to Heribert-Nilsson's interpretation, the progenies consisted only of heterozygotes and recessive homozygotes. The elimination of the hypothetical dominant homozygotes he accounted for by assuming that in certain cases an incompatibility, or, as he puts it, a prohibition, exists between like gametes. His whole hypothesis is based upon this idea of prohibition. He assumes that the assemblage of characters which we recognize in *Œnothera Lamarckiana* may be brought about by many combinations of plural factors. Any one of these plural factors in the heterozygous condition gives a plant the *Lamarckiana* habit, and prohibition prevents the presence of any of them in the homozygous condition. Segre-

gation may lead to the production of pure recessives, lacking all the plural factors which give the *Lamarckiana* aspect. These recessives are the supposed mutations. Pure dominants, on the contrary, can not be realized.

This, in brief, is the Mendelian explanation of mutability. It involves the important assumption that the mutations which breed true are Mendelian recessives. The mutations with irregular chromosome numbers have been shown not to belong in this category. The remaining mutations, for many of which the cytological data are lacking, may conveniently be divided into two classes, (1) those which come true when self-pollinated, or, at any rate, do not include the parent species in their progeny, and (2) those which give a mixed progeny consisting of the mutational and parental forms. If there is any possibility whatever that the Mendelian explanation of mutability is true, it should at least account for the first and simplest of these two cases. We shall therefore confine our attention for the moment to mutations which give a constant progeny.

De Vries found that certain of the original mutations from *Œnothera Lamarckiana* were of the Mendelian type. These mutations are assumed by Heribert-Nilsson to be recessives which have corresponding homozygous dominants, the latter being the strains of *Œ. Lamarckiana* which do not give rise in every generation to the mutations in question. Other mutations, isolated by Heribert-Nilsson himself from *Œ. Lamarckiana*, are produced in every generation, and are therefore, according to this author, recessives which have no corresponding homozygous dominants. If this were the case, they would be recessive when crossed with *Œ. Lamarckiana* regardless of which way the cross was made. As a matter of fact, Heribert-Nilsson made his crosses with *Œnothera Lamarckiana* as the pistillate parent, and therefore obtained the results which he expected. If the crosses had been made the other way, there is very good reason to believe that he would have got the most unexpected results, and would never have advanced his Mendelian hypothesis.

The speaker has recently observed, in several species of *Œnothera* other than *Œ. Lamarckiana,* the origin of a large number of different mutations. Several of these have been found to belong to the type which we are at present considering. That is to say, they give a progeny which does not contain the parent species, and the mutations themselves are produced by the parent species in every generation. In the case of one mutation, described a year ago as *Œ. pratincola* mut. *nummularia,* the chromosome number has been determined as 14, the typical number in the group. The remarkable fact about these mutations of *Œ. pratincola,* as far as work with them has gone, is that their crosses with the parent species are identical with the pistillate parent in the first hybrid generation. Mutation pollinated with parent species yields the mutation. Species pollinated with mutation yields the species.

This most interesting state of affairs is absolutely at variance with the attempted Mendelian explanation. It can be understood on the supposition that two types of gametes are produced, which are by no means equivalent. One type bears most of the characters which differentiate the different species and forms from one another. The other type seems to carry characters which are likely to be common to a number of different species. In the particular species which gives rise to the mutations under discussion the gametes of the former class are female, those of the latter, male. Thus it follows that a mutative modification of the germ plasm in one of these species might affect only characters which were borne by one of the two kinds of gametes. If so, we would have at once a simple explanation of the behavior of the mutations which give matroclinic crosses with their parent species.

The same idea may readily be extended to cover the cases of mutations which give progenies containing both the mutational and the specific types. Perhaps the mutative change is a reversible one, and certain gametes in each generation show reversion from the mutated to the unmutated condition. Or perhaps in some species there

are male and female gametes of both types, but certain mutative changes are sex limited. In the following discussion I shall designate the two types of gametes as α and β gametes. The former are those which bear the most distinctive specific characters of the various forms, whereas the latter bear the more general characters. The known facts seem to be accounted for if we assume that in fertilization the conjugation of an α with a β gamete ordinarily takes place, but not the conjugation of two β gametes. In certain cases it seems that fertilization takes place by the fusion of two α gametes and it appears likely, also, that some species produce no β gametes. Some species produce α and β gametes of both sexes. Others do not seem to do so. It sometimes seems to be the case that the female gametes are all α. When a mutation takes place the modified character is perhaps Mendelian if it is borne by both α and β gametes, but non-Mendelian if it affects only the α gametes of a species in which fertilization takes place by the fusion of an α with a β gamete.

This conception of non-equivalent gametes has been highly developed by de Vries, in a somewhat different way from that outlined above. It has many obvious advantages in explaining the *Œnothera* situation. It explains seed sterility as well if not better than the Mendelian hypotheses of Renner and of Heribert-Nilsson, hypotheses which are based of course upon the idea of gametic equivalence. It explains why certain reciprocal crosses are alike, and others unlike, why some of them breed true, whereas others show segregation, why certain crosses yield twin hybrids, and why the twins are, respectively, matroclinic and patroclinic. It also explains other complications which are quite unintelligible from a Mendelian standpoint. I would by no means give the impression that there are not many phenomena which remain obscure, but I do wish to emphasize very strongly that a flood of light is thrown upon the *Œnothera* situation by the conception of non-equivalent gametes.

By way of illustration, let us consider for a few moments the phenomenon which I have called mass mutation.

Mass mutation differs from ordinary mutation only in that the mutations, instead of being produced in small numbers, are produced in very large numbers. For example, the frequency of mutations in *Œ. Lamarckiana*, which shows ordinary mutability, is roughly 2 per cent. In certain strains of *Œ. Reynoldsii* and *Œ. pratincola*, on the contrary, the number of mutations rises to 50 per cent., or even 100 per cent., of the progenies. According to Mendelian conceptions, it is impossible to get extracted recessives in a progeny in excess of 33⅓ per cent., and in order to get this many we must grant the elimination by prohibition of the corresponding dominants. What shall we say, then, of progenies containing 499 mutations out of 500 plants, a condition which has actually been realized in my cultures of *Œ. pratincola?* It is impossible to invoke the elimination of a large class of typical plants, for the typical zygotes are known to be stronger and better fitted to develop than the mutational zygotes. My own explanation is that most of the female germ cells of *Œ. pratincola* are α gametes and the male, β gametes. The phenomenon of mass mutation consists in the wholesale production of modified α gametes, α', α'', α''', α'''', etc., each of which corresponds to a different mutation and has characters which impress a distinctive habit on the zygote which is formed by fusion with an unmodified β gamete. In accord with this hypothesis the reciprocal crosses between mutation and parent species are matroclinic. Mutation pollinated with species gives mutation. Species pollinated with mutation gives species.

Mention has already been made of the mutations which by self-pollination give progenies containing both the mutational and the specific types. If the mutation is cross-pollinated with pollen from the specific type, the progeny is a mixture of two types, just the same as if self-pollination had occurred. On the contrary, if the specific type is pollinated by the mutation, only the specific type occurs in the progeny. Here, it seems, we have a case where the modification which results in the production of α' instead of α gametes is reversible. Cases of this kind

Heribert-Nilsson refers to (I give a literal translation) as "heterogamous combinations which are recessive only in the female gametes, but in the male gametes continuously heterozygous." As far as I can interpret this vague statement at all, it involves a decidedly unique conception, namely, that the individual $2x$ mutation embodies two different kinds of germ plasm, a homozygous female germ-plasm which will give one kind of cells when the reduction division takes place, and a heterozygous male germ-plasm, which will give two kinds of cells. I think that no one will be inclined to adopt this altogether revolutionary and useless hypothesis. It is by no means certain, after all, that the mutations which show the type of inheritance in question do not belong to the class with irregular chromosome numbers. With one exception they have not been examined cytologically. *Œnothera lata*, a mutation which shows this type of inheritance, has 15 chromosomes. Consequently there is an opportunity for the formation of two kinds of gametes, with 7 and 8 chromosomes, respectively. The male gametes with 8 chromosomes appear to be eliminated. As a result, zygotes are formed with $7 + 7 = 14$ and $8 + 7 = 15$ chromosomes. The former are *Œ. Lamarckiana*, the latter are *Œ. lata*. This beautiful correlation of cytology with inheritance has been worked out by Gates and Thomas.

In either event, whether the mutations which throw the specific type in every generation have a regular or an irregular chromosome number, the mutation hypothesis provides a far more plausible explanation for their behavior than the Mendelian hypothesis.

It must be clear by this time that the speaker finds incredible the arguments that have been brought forward in favor of the idea that mutation and Mendelian segregation are the same. Doubtless it often happens that a mutated germ cell fuses with a typical germ cell and produces an ordinary Mendelian heterozygote. If the mutated character is recessive, and the dominance is complete, the first hybrid generation will of course resemble the parental type, and the second hybrid generation will

show simple segregation. The mutation will appear for the first time in 25 per cent. of the progeny. De Vries has recently reported that the dwarf mutation from *Œnothera gigas* is of the simple recessive Mendelian type. We must believe, in a case of this kind, that the factor whose modification results in dwarfness is present in all gametes. It does not follow, however, that the gametes are all equivalent with respect to the factors for other characters.

In connection with the discussion of Davis's hybrids which resembled *Œnothera Lamarckiana* I mentioned that the mutability shown by them was probably inherited from one or both parents. There seems to be some scepticism about the inheritance of mutability as a character. Much of my own experimental work of the last two years has involved *Œ. pratincola*, a mutable species which has already been refered to several times. There is another species from the same locality which is rather closely allied to *Œ. pratincola*, but differs in enough regards so that the hybrids between them can be studied with great satisfaction. The second species, *Œ. numismatica*, is immutable, as far as my experience extends. At any rate it is very much less mutable than *Œ. pratincola.* The cross *Œ. pratincola* × *Œ. numismatica* gives twin hybrids, one of which is exactly like the pistillate parent except in one minor pubescence character. The reciprocal cross, *Œ. numismatica* × *Œ. pratincola*, is to all outward appearances the same as the pistillate parent. We have here a most striking case of matroclinic reciprocal hybrids. I am inclined to believe that most of the differences between the two species reside in the α gametes and that the β gametes are essentially similar. In accord with this hypothesis nothing could be more interesting than to find that the *pratincola*-like hybrid is mutable, and produces the same types of mutations that *Œ. pratincola* itself does. This result, it seems to me, is of the highest significance. It indicates that the germ plasm of *Œ. pratincola* is in a labile condition, and that this condition is not modified when a zygote is formed by the fusion of its α gamete with the β gamete of a different and stable, or at least relatively

stable, species. We could hardly find better proof that such mutations in *Œnothera* involve the α gametes, and are apparent in the zygotes without the need of subsequent segregation because the factors involved have no counterparts in the β gametes.

The same crosses, however, afford evidence that certain characters are carried by both α and β gametes, and may therefore prove to show Mendelian segregation. The buds of *Œ. numismatica* have a short viscid pubescence which is lacking in *Œ. pratincola*. The matroclinic hybrid *Œ. pratincola* × *Œ. numismatica* can be distinguished from the pistillate parent only by the presence of this hair-type, inherited from the pollen parent. When the second hybrid generation is grown, segregation with regard to this character takes place, and part of the progeny can not possibly be distinguished from *Œ. pratincola*.

In these results we have a clue to the segregation shown in certain hybrids, and the lack of it in others. Most of de Vries's hybrids have involved *Œ. Lamarckiana,* a species, according to my interpretation, with very dissimilar α and β gametes. He has therefore obtained and described many measurably constant hybrids. Davis, however, studying *Œ. grandiflora,* which may conceivably have but one type of gametes, has found segregation the rule rather than the exception. In his later studies, involving *Œ. franciscana* and *Œ. biennis,* he has obtained twin hybrids within each of which there was a considerable degree of segregation. All of these varying results will eventually become coordinated as we become more used to distinguishing between non-Mendelian and Mendelian characters.

Another point which must be mentioned is the frequence with which the various types of mutations give rise to one another. For example, two mutations of *Œ. pratincola,* mut. *nitida* and mut. *fallax*, each give rise to plants of mut. *numularia,* which are as typical as though they had been derived directly from *Œ. pratincola*. As already brought out, some mutations appear to be reversible in that they revert to the parent species in part

of every progeny. The germ plasm seems to be a system capable of existing in several different states of equilibrium. Some of these equilibria may be thought of as stable, others as metastable, others as labile, to borrow terms from the physicist. The germ plasm of different species may undergo parallel transformations, resulting in parallel variations. All who have dealt with the species of large genera know that oftentimes the same series of variations turns up in one collective species after another. Many characters have arisen independently, at so many points in different lines of descent, that they have no phylogenetic significance whatever.

It seems to the speaker that the *Œnothera* situation is clearing up. More and more evidence is accumulating which shows that although the phenomena are complex, they are orderly. Probably no two of the workers on the *Œnothera* problem look at it from the same point of view. In this paper I have not hesitated to state freely my present working hypotheses. Next year they may have changed, to fit new facts. Even now there are data at hand which do not accord with the best hypotheses I have been able to formulate, but neither do they accord with any others. Under the circumstances, one should not draw conclusions of too sweeping a nature. It may confidently be stated, however, that the appearance of mutations in *Œnothera* is not due to Mendelian segregation, and that the Mendelian method of attack has been utterly fruitless. It is freely admitted that the mutation processes themselves are hardly understood at all, and that further work must decide whether or not mutation is always or ever conditioned by previous hybridization.

Bateson has recently described the genetical behavior of the rogues which occur in certain varieties of peas. Although he does not suggest that these strange forms are mutations, his evidence would tend to convince a mutationist that they are. Would it not be a strange turn of fate if Bateson, the leader of the Mendelian school and critic of de Vries, were destined to discover mutations of a non-Mendelian type in the very genus which provided Mendel with the material for his classical researches?

INHERITANCE STUDIES IN PISUM

I. Inheritance of Cotyledon Color[1]

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Introduction

Modern students of genetics such as Baur, East, Morgan, Emerson, and others classify all variations in animals and plants into three general categories on the assumption that organisms are made up of unit factors, in the same way that a chemist thinks of rocks and minerals as being composed of elements. These three categories of variation are:

1. Variation resulting from changes in environment.
2. Variation due to "loss" or "gain" of new factors through crossing.
3. Variation due to mutation.

The Problem

The present paper has to do largely with data on variations in *Pisum* belonging to the first and second categories mentioned above. An attempt is being made definitely to work out the Mendelian or factorial constitution of the genus *Pisum* with reference to all those characters by which its few species and numerous varieties are distinguished. In order satisfactorily to accomplish this object, all or nearly all the known varieties of the genus *Pisum* must be considered. In this paper only the inheritance of cotyledon color is considered. Further papers

[1] Published as Brooklyn Botanic Garden Contributions, No. 10. These studies on the genetics of *Pisum* are being carried on in collaboration with the Office of Forage Crop Investigations and the Office of Horticultural and Pomological Investigations, U. S. Department of Agriculture. Based in part on a paper given at the Twentieth Anniversary Celebration, New York Botanical Garden, September 9, 1915.

will deal with other characters and the modifications of various characters through crossing.

Of more than two hundred and fifty varieties and species upon which the writer has been conducting experiments, the great majority have seeds which in the mature condition possess yellow cotyledons, but in such an array of varieties, it was soon noticed that the shades of yellow varied from a light greenish yellow to that of a deep orange. Roughly one could divide these forms with yellow cotyledons into light and deep yellows, but any one particularly "keen" on forming a series showing continuous variation, could easily grade the varieties so as to present a series without breaks from light greenish yellow to deep orange yellow. All the wild varieties and species so far examined have yellow cotyledons, which favors the assumption that yellow cotyledon is the oldest color character. Many of the cultivated varieties and especially the so-called blue "field peas" such as Wisconsin Blue and Prussian Blue and the majority of those known as "garden peas" have green cotyledons when the seed is mature. What has been stated regarding the gradations of color in yellow cotyledon varieties is equally true of those with green cotyledons. Roughly classified, there are dark and light green forms, but the various varieties can be arranged in a continuous series representing every shade from very dark green to light yellowish green.

Among the numerous green and yellow cotyledon varieties, when grown under the same environment, there are, however, many varieties to which certain distinct shades of either green or yellow are peculiar. Some varieties have characteristically deep orange cotyledons, others have light yellow cotyledons, and still others breed true to the shades between these two extremes. With the group of green cotyledon varieties, the same state of affairs holds true. Classification of yellows and greens is still further complicated because some varieties with light yellow cotyledons grade into the light greens and *vice versa*, even though both are grown under the same conditions.

The following table (Table I) gives the names of varieties of greens and yellows representing the classes dark

TABLE I

VARIETIES OF PISUM CLASSIFIED ACCORDING TO SHADES OF COTYLEDON COLOR WHEN GROWN UNDER THE SAME CONDITIONS

Yellow Cotyledons

Cotyledon Shade	Variety	Pedigree Stock No.	Source
Orange yellow	Black-Eyed Marrowfat	14	Vaughan Seed Co.
	First of All	22	P. Henderson & Co.
	Petit Pois	25	P. Henderson & Co.
	Späte Gold	29	Haage & Schmidt
	Henderson's Perfection Sugar	60	P. Henderson & Co.
	Agnes	147	S.P.I. 22036
	Admiral	159	S.P.I. 29323
	Khaba	176	S.P.I. 20380
Yellow	Mummy	1	H. Eckford, Wem, Eng.
	White Marrowfat	23	P. Henderson & Co.
	Elephanten	31	Haage & Schmidt
	Wachs Schwert	32	Haage & Schmidt
	Gold von Blöcksberg	34	A. D. Darbishire
	"*P. Jomardi*"	40	Cambridge (Eng.) Bot. Gard.
	P. elatius	41	Cambridge (Eng.) Bot. Gard.
	Prosperity	71	P. Henderson & Co.
	Pois géant sans parchemin	107	Vilmorin & Cie
	Abyssinian Black	132	S.P.I.,[2] Dept. of Agriculture
	Pisum formosum	137	H. Winkler
	Openshaw	208	S.P.I. 25439
	Archer	209	S.P.I. 22037
Light yellow	Goldkönig	30	Haage & Schmidt
	Pisum humile ?	33	A. Sutton
	Benton	138	S.P.I. 18396
	Killarney	150	S.P.I. 22078
	Khauaka	165	S.P.I. 31808

Green Cotyledons

Cotyledon Shade	Variety	Pedigree Stock No.	Source
Green to light green to yellowish green	Alaska	15	Vaughan Seed Co.
	Telephone	26	P. Henderson & Co.
	Laxtonian	27	P. Henderson & Co.
	Acacia (wrinkled)	38	W. Bateson
	Everbearing	62	P. Henderson & Co.
(Fade easily)	Yorkshire Hero	65	Thorburn & Co.
	Duke of Albany	93	Sutton & Sons
	Hundredfold	97	Sutton & Sons
	Alfred	140	S.P.I. 12888
	Blue Prussian	154	S.P.I. 19787
Yellowish green	Acacia (wrinkled)	38	W. Bateson
	Duke of Albany	93	Sutton & Sons
	Hundredfold	97	Sutton & Sons

Dark green	Market Split Pea	35	New York City Markets
	Acacia (Smooth)	39	W. Bateson
	Velocity	59	Vaughan Seed Co.
	Braunschweiger	88	Haage & Schmidt
	French Grey	149	S.P.I. 27003
	Rosenberg	161	S.P.I. 10274
	Alaska	193	S.P.I. 29366
	Scotch Beauty	198	S.P.I. 27004
	Wisconsin Blue	207	S.P.I. 22049
Green	Express	20	A. D. Darbishire
	Nott's Excelsior	21	P. Henderson & Co.
	Aldermann	28	Haage & Schmidt

yellow, yellow, light yellow, dark green, green, light green, and yellowish green, when these varieties are all grown under approximately the same conditions. Any one can distinguish between dark green and dark yellow, but one well acquainted with the color of cotyledons in *Pisum* would have difficulties in distinguishing between light yellowish greens and light yellows. The classification made is admittedly arbitrary, though based on the same sort of acquaintanceship with these colors as that of a nurseryman with varietal differences in bulbs or varietal characters in leafless nursery trees. The point which it is desired to emphasize by the foregoing remarks is that these shades of cotyledon color are distinctly *varietal* characters, and are always characteristic of the respective varieties when these varieties are all grown together under any one of the several specific[3] environments in which the pea cultures at the Brooklyn Botanic Garden have been grown.

The Relation of Environment to Cotyledon Color

One other perplexing factor enters into the study of cotyledon color in *Pisum*—the difficulty of being certain that all varieties under observation mature their seed under as nearly as possible identical environments, a factor that many geneticists experimenting with other plant forms are prone to neglect. Some varieties of peas

[2] S.P.I. stands for Office of Foreign Seed and Plant Introduction, U. S. Department of Agriculture, to which I am very much indebted for help in collecting varieties and species of the genus *Pisum*.

[3] These environments will be described in detail in a later paper.

gradually change from green to yellow when maturing, while others appear to change very suddenly, but only if plenty of sunlight and no over-supply of moisture is present. This is particularly true of some of the deep orange varieties, such as Späte Gold (P 29). The seed of this variety remains very dark green until the general appearance of the vine leads you to suppose it is ripe, but if plenty of sunlight is present and not too much moisture, and the pods are allowed to remain for a few days, the dark green changes to a very deep orange, and this deep orange is characteristic of Späte Gold when grown commercially.

With the green cotyledon varieties, one is bothered by fading of the green to a sort of washed-out yellow in many varieties, if the vines are not harvested at exactly the right time. Express, Velocity and many of the wrinkled sorts (see Hurst, 1904) are particularly subject to change under these conditions.

The above mentioned difficulties regarding the proper maturing of pea seed have been considered quite fully by Bateson, Darbishire, Lock, Tschermak and other workers in genetics. Hurst (1904) and Lock (1905) particularly have studied the tendency of certain varieties such as Telephone with green cotyledons to fade easily, even when harvested carefully, but left exposed to light. Bateson and Kilby (1905, p. 58) have studied the so-called "piebald" peas (peas with green or yellow cotyledons partly spotted or tinged with both colors) and find them to largely result from environmental conditions such as failure to ripen properly or from bleaching after ripening. "Piebald" peas are characteristic of certain varieties of peas, in which the green fades much faster upon exposure to light or moisture or to both than in ordinary green cotyledon types. "Piebald" peas of one pod, according to Bateson and Kilby, are always tinged on the same surface. Injuries causing the death of the cotyledon tissue (Bateson, 1905) (Tschermak, 1902) also are a cause of yellow spots on peas from green cotyledon varieties.

The Pigments of Cotyledon Color in Pisum

Bunyard (see Darbishire, p. 131) has shown that both yellow and green cotyledon varieties have a yellow and a green pigment in their cotyledons when the seed is immature, but the yellow cotyledon varieties possess a factor (an enzyme perhaps), which causes the green pigment to fade on the maturity of their seeds. Thus green pigment is epistatic to yellow pigment, since, when both are present, only the green is in evidence.

The Genetics of Cotyledon Color in Pisum

Historical

As early as 1729, according to Darwin (1876, I, p. 428) white (yellow cotyledon) and blue (green cotyledon) peas were found in the same pod and these results were understood to be due to chance crossing. Wiegmann, Goss (1824) and others observed that varieties of *Pisum* breeding true to blue peas when crossed with pollen from varieties breeding true to white peas, always showed a direct and immediate effect of the pollen parent. Gärtner (1849) and later hybridists incorrectly regarded this phenomenon as xenia, believing that tissues of the parent generation were affected so that the color of the seed was changed. The fact that the change in color was due to an embryonic character of a new hybrid generation seems never to have occurred to them. The true significance of these facts were never understood by Knight, Goss, Gärtner, nor any of the hybridizers before Mendel's time. Knight distinguished between cotyledon colors and seed coat colors, and Goss and others had observed practically everything regarding crosses between green cotyledon and yellow cotyledon peas except the numerical proportion of one to the other in the F_2 generation. Darwin (1876, p. 348) mentions some observations of Masters, which, if authenticated, show a complex state of affairs in the inheritance of cotyledon color, since Masters claims to have obtained both yellow (white) and green (blue)

peas from a certain pea plant and when these two kinds were planted separately each continued to produce the two kinds through four generations, that being as far as the experiment was carried. In the light of the data I present below his observations may be correct, he having possibly secured one of the yellow forms such as I have found.

Mendel (1865) found when peas with yellow cotyledons were crossed with green cotyledon forms that the first generation offspring all had yellow cotyledons, but each one of these yellow cotyledon F_1 plants produced F_2 seeds, approximately three fourths of which had yellow cotyledons and one fourth green cotyledons. Either color of parent could be used as the seed or female parent, and the result was the same. Further, the F_2 greens in F_3 only produced greens, while the F_2 yellows when planted, in some cases gave only yellows, in other cases both yellows and greens in the proportion of 3 Y : 1 G. The actual data by which Mendel supported these statements are as follows: fifty-eight crosses on 10 plants were made, and in every case, yellow was dominant to green in the F_1 generation of these crosses. 258 F_1 plants produced 8,023 F_2 seeds of which 6,022 were yellow and 2,001 had green cotyledons, an actual ratio of 75.1 yellow to 24.9 green or 3.01 Y : 1 G. Mendel is careful to call attention to the wide variability in the ratio of yellows to greens when the F_2 peas of each F_1 plant are considered separately, the variation ranging from 32 Y : 1 G on one plant to 20 Y : 19 G on another. Between these extremes, there were some among the 10 F_1 plants of which he gives the ratios, that closely approximated the theoretical 3 : 1 ratio. I call attention to this great variability that Mendel found because some geneticists of late, apparently not having noted that Mendel himself observed these same facts, have referred to this as a new phenomenon. Only average ratios from large numbers were considered by Mendel, as small numbers tended to obscure the significance of the facts. Of the 8,023 F_2 seeds secured by Mendel,

519 seeds with yellow cotyledons were used to grow an F_3 progeny. Of these, 166 F_2 seeds bred true or produced only seeds with yellow cotyledons, while 353 produced both yellows and greens in the proportion of 3 Y : 1 G. 353 to 166 gives a ratio of 2.13 to 1. Mendel (p. 327) especially calls attention to the difficulties involved in classifying the two colors of seeds, and notes, as I have done in the preceding paragraphs, that the seeds of pure green varieties and of segregate greens, have a tendency to bleach, another fact that several critics of Mendelian methods seem to have overlooked or forgotten.

Mendel's work has been substantiated by a large number of trained investigators, as well as by a host of teachers and amateurs. The results for cotyledon color in *Pisum* obtained by seven well-known geneticists are given below (Table II).

TABLE II

Hybrid Generation	Observer	Yellow	Green	Percentage of Green
Second.........	Mendel	6,022	2,001	24.9
	Correns	1,394	453	24.5
	Tschermak	3,580	1,190	24.9
	Bateson	11,903	3,903	24.7
	Hurst	1,310	445	25.4
	Lock	1,438	514	26.2
	Darbishire	1,089	354	24.9
Third..........	Correns	1,012	344	25.5
	Tschermak	3,000	959	24.2
	Lock	3,082	1,008	24.6
	Darbishire	5,662	1,856	24.7
Fourth.........	Correns	225	70	23.7
	Lock	2,400	850	26.1
Total..........	56,064	42,117	13,947	24.9

These results approximate very closely the ratio of 3 Y : 1 G demanded by Mendel's theory. Darbishire (1913, p. 62) in testing out 140 F_2 progeny with yellow cotyledons, secured 98 F_3 plants heterozygous for green and yellow cotyledons, and 42 breeding true or homozygous for yellow cotyledons, a proportion of 2.3 heterozygous F_2 plants to 1 homozygous F_2 yellow. Many varieties gathered from all over the world were used in these

[4] These data are taken from Darbishire (1913).

studies and all gave similar results. With these facts before us, there can be no denying the validity of Mendel's law as regards inheritance of cotyledon color in *Pisum*. The criticism has sometimes been made that the F_2 segregate yellows and greens were a little less green and a little less yellow owing to the association of the unit factor materials of the two pigments in the F_1 generation. In other words, segregation was not complete; the

TABLE IIIa

CROSSES OF DOMINANT YELLOW WITH GREEN (F_2 GENERATION)[6]

Crosses	Cotyledon Color Yellow	Green
(P1–4 × P39–3)–1	76	34
(P21–1 × P1–1)–1	129	41
(P23–5 × P39–3)–1	60	16
(P26–1 × P1–1)–1	23	7
(P26–1 × P1–1)–2	16	0
(P26–1 × P1–1)–3	32	10
(P26–1 × P1–1)–4	34	6
(P27–1 × P1–1)–1	33	10
(P28–1 × P1–1)–1	48	6
(P28–1 × P1–1)–2	37	22
(P28–2 × P1–2)–1	46	17
(P29–1 × P35–7)–1	11	4
(P32–1 × P35–2)–1	65	16
(P34–2 × P35–7)–1	103	34
(P35–3 × P1–4)–1	9	4
(P35–3 × P1–4)–2	49	16
(P39–1 × P32–1)–1	63	17
(P40–1 × P59–1)–1	91	30
(P40–1 × P59–1)–2	68	29
(P41–1 × P21–1)–1	103	30
(P41–1 × P21–1)–2	81	25
(P62–1 × P41–3)–1	40	17
(P62–1 × P41–3)–2	74	22
(P65–1 × P41–3)–1	71	29
(P72–1 × P41–5)–1	143	54
(P72–1 × P41–5)–2	142	47
Total actually obtained	1,647	543
Total—theoretically expected	1,642.4	547.5
Ratio (theoretical)	75 yellow	25 green
Ratio (actually obtained)	75.2 yellow	24.8 green

[6] Pedigree numbers such as –1, –2, etc., following the pedigree stock number of the variety as, *e. g.*, P28–1 refer to plant numbers. P28–1, *e. g.*, is progeny plant No. 1 of stock variety P28. P28–1–1 is plant No. 1 of the second generation from pure inbred stock P28. The seed or maternal parent in a cross is always given first, *e. g.*, P28–1 ♀ × P29–1 ♂.

determiner for green pigment was not able to produce as dark a green in F_2 green segregates as in peas of the green cotyledon parent race. This is true undoubtedly in some few cases, but in still others, Hurst (1904), Darbishire (1913) and myself have been unable to find any distinction in shading by comparing the segregates with the grandparental seeds of both colors. In those cases where there has been found a difference, the observers probably failed to take into account all the environmental factors.

New Data

In my own investigations[5] on the heredity of cotyledon color, the F_1 and F_2 generations from over 79 crosses involving combinations of 40 varieties and species of *Pisum* have given results similar to those secured by other workers except in the case of crosses involving a variety of German pea, "Goldkönig," obtained from Haage & Schmidt. The data for most of these crosses are given in Tables III*a*, III*b*, III*c*.

TABLE III b

Crosses of Dominant Yellow and Recessive Yellow (F_2 Generation)[7]

Crosses	Cotyledon Color: Yellow	Cotyledon Color: Green	Cotyledon Color: Yellowish Green
(P22–3–1 ×P30–A–5)–1	26	7	
(P22–3–1 ×P30–A–5)–2	18	6	
(P22–3–1 ×P30–A–5)–3	11	3	
(P22–6–1 ×P30–A–3)–1	17	5	
(P30–1 ×P1–1)–1	73	16	2
(P30–2 ×P1–1)–1	51	5	6
(P30–2 ×P1–1)–2	62	8	3
(P30–3 ×P32–1)–1	52	10	3
(P40–2 ×P30–4)–1	98	15	7
(P41–1 ×P30–6)–1	49	12	1
Total—actually obtained	457	87	22
		109 (green + yellowish green)	
Total—theoretically expected	459.2 yellow : 106.2 green		
Ratio	13 yellow : 3 green		

[5] All varieties of peas have been inbred for at least two generations and all the ordinary precautions against differentiating environmental factors, insect pollination, etc., in use by geneticists have been employed.

[7] The investigations of Mendel, Bateson, Lock, Tschermak and others

TABLE III c

CROSS OF GREEN × RECESSIVE YELLOW (F_2 GENERATION)[8]

Cross	Yellow or Yellowish	Green
(P21–15–1 × P30–A–2)–1	2 + 1?	12
(P21–15–1 × P30–A–2)–2	4 + 1?	22
(P30–5–4 × P38–20–1)–1P	15 + 1?	30
(P30–5–4 × P38–20–1)–2P	15	13 + 4?
(P30–5–4 × P38–20–1)–1	2 + 2?	16
(P30–5–1 × P38–20–1)–1	7 + 1?	26
(P35–1 × P30–3)–1	9	22
(P35–1 × P30–3)–2	0	20
(P35–9–1 × P30–5–4)–1	1	6
(P35–9–1 × P30–5–4)–2	4	29
(P35–9–1 × P30–5–4)–3	1 + 1?	8
(P35–10–2 × P30–5–6)–1	1	12
(P35–10–2 × P30–5–6)–2	1 + 1?	26
(P35–10–2 × P30–5–6)–3	0	10
Total actually obtained, 326....	70	256
Total theoretically expected	81.5	244.5
Ratio	1 yellow	: 3 green

The variety "Goldkönig" breeds true to yellow cotyledons and wrinkledness. When crossed with varieties breeding true to green cotyledons, the F_1 generation was invariably green. In most of the crosses, the seed parent was the green variety, but reciprocals have been obtained in two cases.

The crosses were:

Goldkönig × Acacia and reciprocal

Goldkönig × Market Split Pea and reciprocal

have always shown cotyledon color in peas to be inherited independently of roundness and wrinkledness of cotyledons. The data given in Tables III b and III c give reason to believe there is linkage or partial coupling involved. Round yellow × wrinkled yellow (Goldkönig) gives practically only three classes in F_2—round yellow, wrinkled yellow, and round green. All four classes appeared in only one cross, where a single wrinkled green was obtained. Round green × wrinkled yellow (Goldkönig) gave all the expected classes except round yellow, which was absent from the F_2 progeny of all the eight crosses examined. Wrinkled yellow (Goldkönig) × wrinkled green and reciprocal gave wrinkled yellows and wrinkled greens approximating the expected ratio.

[8] The cotyledon colors of the peas concerned in Tables III b and III c were for the most part independently determined by two separate people, and these determinations when compared, differed but slightly, and only in very few cases. For help in these determinations, I am indebted to Mr. Montague Free of the Brooklyn Botanic Garden staff.

Nott's Excelsior × Goldkönig
Aldermann × Goldkönig
Scotch Beauty × Goldkönig
Sutton's Main Crop × Goldkönig

An F_2 generation has been grown from the first three of these crosses with the results (see Table III*c*) approximating a ratio of 3 G : 1 Y or the reverse of the common result. Practically all of the green seeds are distinct greens, but among those classed as yellows are several doubtful cases, and these are marked questionable. An F_3 generation is being grown which will decide whether I have erred in considering these doubtful cases as yellow cotyledon peas in which the greenish color results possibly from lack of enough sunlight during the ripening period.

When the "Goldkönig" yellow was crossed with other varieties having yellow cotyledons, the F_1 progeny all had yellow cotyledons, but in the F_2 generation, a certain proportion of peas with distinctly green cotyledons appeared, the F_2 ratio in the progeny of the ten different F_1 plants, showing considerable variation, but averaging 13 yellow seeds to 3 green seeds, provided all yellows having any considerable amount of green pigment are classified as greens. The number of F_2 generation progeny obtained was small, totaling only 566, of which 457 had yellow cotyledons, 87 distinctly green cotyledons and 22 seeds had yellowish green cotyledons. All were grown under conditions insuring their maturity, but under these conditions (greenhouse cultures) the amount of moisture present is such as possibly to cause some of the true greens to bleach. Further justification for classifying the 22 doubtful greens as true greens comes from the fact that all the varieties having yellow cotyledons used in these crosses with the exception of Goldkönig are varieties with distinctly bright yellow cotyledons, which grown under the same conditions and often side by side with the crosses, *never* show any green coloring matter in the coty-

ledons of their mature seeds. The crosses of dominant yellow with Goldkönig were:

"*Pisum Jomardi*" × Goldkönig
Goldkönig × "Mummy Pea"
Goldkönig × Wachs Schwert
"*Pisum elatius*" × Goldkönig
First of All × Goldkönig
Gold von Blöcksberg × Goldkönig
Späte Gold × Goldkönig
Benton × Goldkönig

All the yellows other than Goldkönig yellow gave the ordinary Mendelian ratios when crossed with varieties having green cotyledons. No greens were obtained from crosses between varieties having yellow cotyledons, other than those with Goldkönig.

Theoretical Interpretation

Interpreted in Mendelian terms the above data are brought into accord with other data on the inheritance of cotyledon color in *Pisum* by regarding all varieties of peas, both with yellow and with green cotyledons, as possessing a factor for yellow pigment (Y), while the dominant yellow varieties possess a factor for green pigment (G) and a factor (I) which causes the green pigment to fade on the maturity of the seed. The German variety "Goldkönig" may be regarded as lacking both the factor for causing green pigment and the factor for causing that pigment to fade on the maturity of the seed, while the green varieties lack only the factor (I). Green pigment masks yellow pigment, hence may be regarded as epistatic to yellow pigment.

Regarded thus:

(1) YYGGII = dominant yellow varieties
(2) YYggii = recessive yellow varieties
(3) YYGGii = green varieties

Crossed with each other these give:

$(1 \times 2) =$ YYGgIi (F_1) yellow, (F_2) 13 Y : 3 G
$(1 \times 3) =$ YYGGIi (F_1) yellow, (F_2) 3 Y : 1 G
$(2 \times 3) =$ YYGgii (F_1) green, (F_2) 1 Y : 3 G

The hereditary substances responsible for yellow pigment, of course, have not been isolated and may take the form of more than one factor, but I have represented these as Y, to make my interpretation clearer. The essential point in the interpretation is that all the hereditary differences in cotyledon color in *Pisum* so far discovered may be pictured as due to the presence or absence of tw genetic factors.

TABLE IV

FACTORIAL COMPOSITION OF F_2 PLANTS OF THE THREE CROSSES AND THE APPEARANCE OF THE F_3 PROGENY

Dominant Yellow × Recessive Yellow and Reciprocals

F_2 Ratio	13 Y : 3 G	Character of F_3 Progeny
9 yellow	1 YYGGII	...... Breeds true to yellow cotyledons
	2 YYGGIi	...... 3 Y : 1 G
	2 YYGgII	...... Breeds true to yellow but heterozygous for G
	4 YYGgIi	...... 13 Y : 3 G
3 green	1 YYGGii	.... Breeds true to green cotyledons
	2 YYGgii	.... 1 Y : 3 G
3 yellow	1 YYggII	.... Breeds true to yellow cotyledons
	2 YYggIi	.... Breds true to yellow but heterozygous for I
1 yellow	1 YYggii	.... Breeds true to yellow cotyledons

Dominant Yellow × Green and Reciprocals

F_2 Ratio	3 Y : 1 G	Character of F_3 Progeny
3 yellow	1 YYGGII	...... Breeds true to yellow cotyledons
	2 YYGGIi	...... 3 Y : 1 G
1 green	1 YYGGii	.... Breeds true to green cotyledons

Recessive Yellow × Green and Reciprocals

F_2 Ratio	1 Y : 3 G	Character of F_3 Progeny
1 yellow	1 YYggii	.... Breeds true to yellow cotyledons
3 green	1 YYGGii	.... Breeds true to green cotyledons
	2 YYGgii	.... 1 Y : 3 G

Regarded thus, the F_2 plants of all crosses so far made in *Pisum*, involving cotyledon color, can be represented by the gametic formulæ given in Table IV. The char-

acter of the F_3 progeny, *providing this interpretation of the facts regarding cotyledon color in Pisum holds,* is also indicated in this table.

Additional data on the inheritance of cotyledon color in *Pisum* will be given in a succeeding paper.

CONCLUSIONS AND SUMMARY

Variation in cotyledon color in *Pisum* belongs to all three of the categories of variation mentioned in the forepart of this paper, although there are no definite data as regards the origin of the green cotyledon and the "recessive" yellow cotyledon varieties.

1. Variations in cotyledon color due to environment are:

(*a*) Yellow cotyledon varieties producing seeds with green cotyledons, because of immaturity, absence of sufficient sunlight, excess moisture at the period of ripening of the seed, etc.

(*b*) Green cotyledon varieties, especially those with wrinkled seeds, producing seeds which fade or bleach to yellow or yellowish green owing to excess of moisture and sunlight after the seed has matured.

2. Variations due to innate or hereditary differences probably arising as mutations are:

(*a*) Different degrees or intensities of yellow and green coloring in the different varieties of *Pisum*. These different intensities are characteristic of particular varieties when all varieties under consideration are grown under approximately the same environment.

3. Hereditary distinctions as regards cotyledon color in *Pisum* may be represented by the presence and absence of two factors, a factor (I) causing green pigment to fade when the variety matures its seed, and a factor (G) causing the production of green pigment. All varieties of *Pisum* so far experimented with, have yellow pigment in their cotyledons and the determiner or determiners responsible for this pigment may be graphically represented by (Y). As the presence of green pigment masks

yellow pigment, green may be regarded as epistatic to yellow.

4. The majority of varieties with yellow cotyledons when crossed with varieties having green cotyledons, have yellow cotyledon F_1 offspring, the F_2 generation breaking up into yellow and green cotyledon plants in the ratio of 3 Y : 1 G.

The yellow cotyledon variety "Goldkönig" when crossed with green cotyledon varieties has green cotyledon F_1 offspring, in F_2 giving a ratio of approximately 1 Y : 3 G, just the reverse of the ordinary result.

"Goldkönig" crossed with other varieties having yellow cotyledons has yellow cotyledon F_1 offspring, in F_2 giving a ratio of approximately 13 yellow seeds : 3 green seeds.

With these facts in view, "dominant yellows" may be represented by the formula YYGGII, "recessive yellows" (Goldkönig) by the formula YYggii, and green cotyledon forms by the formula YYGGii. These formulæ account for all the facts so far discovered in experiments on the inheritance of cotyledon color in *Pisum,* except the data on linkage or coupling, referred to in page 539, note 7. These results will be discussed when more data are available.

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THE RESULTS OF FURTHER BREEDING EXPERIMENTS WITH PETUNIA

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In view of the present discussion on the inheritance of doubleness in Petunia[1] and pending further investigation of the evidence in favor of an explanation based wholly or in part upon selective sterility such as has been described by Geerts[2] for *Œnothera Lamarckiana,* by Belling[3] for the Velvet "Bean" (*Stizolobium deeringianum* and other species) and by East[4] for species of *Nicotiana,* it seems desirable to make available the further results of the breeding experiments which have been carried out since the publication of the earlier data in 1910.[5] The two main facts established by the earlier work were

(1) That singles of the cultivated forms of *P. violacea, P. nyctaginiflora* and of various garden strains (Countess of Ellesmere, *hybrida grandiflora* and others) give only singles when self-fertilized or crossed with pollen of other singles.

(2) That these same singles give a mixture of singles and doubles in F_1 when crossed with the pollen of a double.

The later experiments carried out on the same lines and with the same kind of material have considerably widened

[1] Frost, "The Inheritance of Doubleness in *Matthiola* and *Petunia,*" AM. NAT., Vol. XLIX, No. 586, p. 623, Oct., 1915; also Saunders, "Selective Partial Sterility as an Explanation of the Behaviour of the Double-throwing Stock and the Petunia," *Ibid.*, Vol. L, No. 596.

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[5] Saunders, "Studies in the Inheritance of Doubleness in Flowers," I. Petunia. *J. of Genetics,* Vol. I, No. 1, p. 57, 1910.

the basis upon which these generalizations rest; while the fact that mixed F_1 families have now been obtained in the case of a new wild form as well as with one *nyctaginiflora* individual raised from wild seed, though not *proving* that the second statement would invariably hold good for singles of each of these two species, certainly increases the probability that this may be found to be the case.

With respect to these more recent experiments I was able in 1911 to obtain a larger series of counts than had been possible heretofore. For the opportunity to carry out the work on this larger scale I was much indebted to Professor Bateson, who kindly had some 5,000 plants grown for me at the John Innes Horticultural Institution. The results may be summarized shortly as follows:

Seven crossbred singles out of matings in which the four strains mentioned above were variously combined, were self-fertilized. *Only singles were obtained,* viz., 1,200, 123, 89, 73, 33, 32, and 12 in the different families. Total 1,562 singles.

Fifteen singles were tested by crossing and pollen from 8 doubles was used to fertilize them. The single parents included

One *violacea* plant (commercial material, new stock).

Twelve F_1 plants the offspring of 4 singles (Countess of Ellesmere) which had been crossed with pollen from various doubles (*hybrida grandiflora*). Among these 12 were 4 of those which had yielded all-single families when self-fertilized.

One *hybrida grandiflora* plant derived from a mating between two singles each of which was the offspring of a single crossed with pollen from a double.

One F_2 plant derived from an F_1 single out of the mating *nyctaginiflora* × *hybrida grandiflora* (double), the F_1 plant having been crossed back with another double.

Thus doubleness was known to have been introduced into the pedigree only once in the case of the first-mentioned (*violacea*) seed-parent, thrice in the case of the

last (F_2) plant. In the remaining 13 cases it was introduced twice,—in consecutive generations in the case of the group of 12 plants, with the skipping of a generation in the remaining instance. *Families were raised from each of these 15 plants and doubles occurred in them all.* The numbers obtained, as was to be expected from the more extended scale of the experiments, indicate a more uniform proportion of singles and doubles than was apparent in the earlier results. In many families the numbers clearly suggest a ratio of equality; in a few, however, there was a considerable excess on the side either of the singles or of the doubles. No connection could be traced between the proportion of doubles obtained and the number of times doubleness was introduced into the pedigree. The numbers in each family are shown below where the 15 seed-parents are indicated by the capital letters A to O and the pollen parents by the small letters a to h.

Parents	A×a	B×a	B×b	B×c	C×b	D×a	D×b'	E×b	F×a	F×b	F×d	F×e	G×a	G×b	G×c
F_1															
Singles	27	104	74	19	27	99	58	53	250	237	148	63	103	113	78
Doubles	36	63	67	47	22	100	56	51	216	256	207	42	65	63	106

Parents	G×e	G×f	H×d	I×a	J×a	K×g	L×e	L×f	M×c	N×f	O×a	O×b	Totals
F_1													
Singles	25	188	23	58	15	34	20	25	38	51	52	17	1,999
Doubles	40	101	25	78	14	29	17	30	13	39	39	15	1,837

How far these proportions are determined, as suggested by Frost,[6] by a condition of partial selective sterility can hardly be profitably discussed until further microscopic investigations have been made, but that this explanation forms a part, though possibly not the whole, of the explanation appears highly probable. At the time it seemed advisable to postpone further breeding until wild material was available for comparison. Though repeated efforts have been made to obtain seed of wild plants, they have, in the case of *P. violacea*, been so, far quite unsuccessful. In the course of 1912 and 1913 how-

[6] *Loc. cit.*

ever, through the assistance of the authorities at Kew and of Sir Reginald Tower in Argentina, to whom I am much indebted, seed was obtained of wild plants of *P. nyctaginiflora* and also of two new unnamed wild forms (species, both white-flowered), all of which had been collected most kindly by M. Thays, director of the Botanic Garden at Buenos Aires. An entirely new stock of double material differing in nature as well as in origin from that previously employed was also now available. This was raised from

(1) seed of a plant exhibited at the Conference on Genetics held in Paris in 1911, which had some flowers double and others apparently of a normal single structure. This plant had appeared in the grounds of the establishment of MM. Vilmorin-Andrieux et Cie at Verrières-le-Buisson. Some seed harvested from the single flowers, together with a small quantity obtained from the doubles was later most courteously forwarded to me by Dr. Haagedoorn;

(2) seed sent to me by Mrs. Francis of Ventura, California, of an interesting new strain of seed-producing doubles which she had succeeded in raising.[8]

The South American seed samples gave plants of uniform type in the case of the two new forms, each presumably being a distinct species. One of the two (referred to below as P.x.), the seed of which had been collected in Cordova, was crossed with pollen from a double raised from the seed of one of the single flowers on Haagedoorn's half-and-half plant, and also with two seed-giving doubles of the Ventura strain. *Each F_1 family showed a mixture of singles and doubles.* In the case of the former cross a large number of semi-double plants with the supernumerary petaloid structures small or few in number, were also obtained. The numbers recorded are given on page 552.

These 5 seed-parents as well as other individuals tested proved quite self fertile and set a good quantity of seed when self-pollinated.

7 For an account of this strain see Mrs. Myrtle Francis [Shepherd] on "Double Seeding Petunias," *J. of Heredity*, Oct., 1915.

Single Parent	Double Parent	F₁		
		Flowers Magenta		
Flowers White	Flowers Magenta	Singles	Semi-doubles	Doubles
P.x. 1....	Descendant of Haagedoorn's plant....	15	54	23
P.x. 2....	" " "	12	32	15
P.x. 3....	" " "	5	32	4
Totals.	....	32	118	42
	Flowers Rose Pink	Flowers White with Dark Tube		
P.x. 4....	Ventura plant 1....................	14	—	15
P.x. 5....	" " 2....................	3	—	2
Totals.	....	17	—	17

Thus we have evidence that in a third species (for as such there seems good ground for regarding this wild type) doubles appear in F_1 when a cross is made with a double form. The behavior in this respect of the other new form has not yet been ascertained.

With regard to *P. nyctaginiflora* the seed of which was collected from Punta Ballena, Maldonaldo (Uruguay), it was noticed that the plants were not entirely uniform in color, some showing a very definite tinge of purple on the outer side of the flower tube, others scarcely a trace. Similar variations had also been observed in the original (1906) commercial material. Whether this variability is normal to the species or is an indication of crossing is not certain. It is somewhat remarkable that in this original material all the 8 individuals tested proved to be self fertile and yielded abundance of seed, whilst six self-pollinated flowers on three of the Uruguay plants taken at random did not yield a single seed. It seems unlikely that this result could be due to accident, or to a difference of conditions due to the fact that the 1906 plants were grown in the open, whereas the individuals grown from the wild seed were kept in pots in a cool house. Indoor treatment had not been found to affect the fertility of the other strains, and it is hardly likely that it did so here; but this point is now being verified. Only one Uruguay plant was crossed with pollen from a double (Ventura 2). From this cross the same result was ob-

tained as with the commercial material. *F_1 was mixed,* the numbers recorded being 225 singles and 113 doubles.

So far, then, the material employed has furnished no exception to the statement that singles crossed with the pollen of doubles yield some doubles in F_1 though breeding true to singleness when self-fertilized or pollinated by other singles.

Unfortunately very little evidence is yet available as to the results of using the double plant as the seed-parent. Haagedoorn's plant was of an exceptional character and the Ventura plants, though more typical, showed considerable sterility. Only 5 plants were raised from the seed sent by Mrs. Francis. These were all double. Only one individual was obtained by self-fertilization of these plants and this was also double. A cross with a single (Countess of Ellesmere) produced only one offspring and this plant was lost before flowering. It is however hoped by a repetition of this mating to obtain an F_1 generation which will throw further light on the relation of the double to the single.

The following information received from Professor Bateson concerning a cross made at the John Innes Horticultural Institution by E. J. Allard unfortunately only came to hand after the above account had been forwarded for publication. In the mating in question a *nyctaginiflora* plant, one of a batch raised from a sample of the same wild seed as that from which my own plants were grown, was crossed with pollen from a pink-flowered double, a florist's highly cultivated type of plant. *More than 20 plants were raised in F_1 and all were single.* If we consider this number large enough to be taken as conclusive we should have in this experiment an exception to the results hitherto obtained and summed up in the general statement formulated above. We must then suppose that there exist in these *Petunia* forms certain singles and doubles the relation between which is such that doubleness completely disappears in the F_1 generation obtained from a cross between them.

INHERITANCE OF SEX IN THE GRAPE[1]

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SINCE the discovery of Correns in 1907 that in *Bryonia* the staminate plants produce two kinds of gametes with respect to sex, and the pistillate and hermaphrodites only one, great advances have been made in the study of sex inheritance.

Shull (1910, '11, '14) has shown that in *Lychnis dioica* also the staminate plants are heterozygous for the sex genes, while the pistillate ones are homozygous, but that the hermaphrodites are heterozygous for the determiner for femaleness and for the hermaphrodite condition. These hermaphrodites were apparently developed from staminate plants as they bear only partially developed pistils in many flowers, and further, the factor for narrow leaves is linked with the determiner for the hermaphroditic condition, while in the normal males it is linked with that for maleness, as pointed out by Shull. Apparently the determiner for femaleness is carried suppressed and linked with the determiner for maleness in the staminate plants.

A simpler case of sex inheritance than either of the above is that of the sweet pea in which Bateson has shown that genotypically three kinds of plants may be produced; namely, the normal hermaphrodites, which produce only hermaphrodites when selfed, the pistillates bearing contabescent anthers, which when pollinated with pollen from the normal hermaphrodites produce the third type, which is phenotypically the same as the normal hermaphrodites. These when selfed produce hermaphrodites and pistillate plants in a 3:1 ratio, showing them to be heterozygous for

[1] Presented before the Society for Horticultural Science, Ohio State University, December, 1915.

the hermaphrodite and female determiners, and showing further that in the sweet pea only one dose of maleness is necessary for the production of functional stamens.

Similarly in many animals, it has been proved by Wilson, Morgan and others that the males are heterozygous for the sex determiner and the females homozygous, and that this condition is correlated with the presence of two chromosomes in the female which are distinct from the others and which they called the "X" bodies, while in the male only one may be present, or if there are two, the second is sometimes smaller and is spoken of as the "Y" body. Occasionally the X and Y bodies may be of equal size and the supposition that they are different is based upon inheritance studies.

The reverse of the above mentioned condition may exist, as in *Abraxis,* pigeons, cultivated fowl, etc., in which the males are apparently homozygous for the sex determiner and the females heterozygous.

Strasburger found that in *Bryonia* there were two chromosomes which were larger than the others and thought that these might carry the determiners for sex.

A great deal of evidence has recently been collected which points to the chromosomes as being the carriers of factors and as many factors have been shown to be linked with sex, it seems safe to conclude that certain chromosomes carry the determiner for sex. If this is the case, then in the hermaphroditic plants it must be assumed that the determiners for maleness and femaleness are linked or carried in the same chromosome; otherwise there would continually be produced not only hermaphrodites but staminate and pistillate plants as well.

The trend of development in many plant groups seems to be toward the production of a diecious condition by the suppression of the stamens in one set of individuals and of the pistils in another. As an instance of this may be cited the strawberry, in which staminate, pistillate and perfect flowers are produced. The grape and maple and many other plants show a like suppression in varying degrees.

Because of the number of flower types, and the fact that most of our cultivated grapes are only one or two generations from the wild, they would seem to furnish ideal material for the study of sex inheritance.

Two types of vines are found in the wild, those producing functionally pistillate flowers, but bearing reflexed non-functional stamens, and those producing functionally staminate flowers, but bearing suppressed pistils.

We have then apparently a transitional form, in the case of the grape, from a hermaphroditic condition such as is found in the apple, in which the male and female determiners are apparently linked, to the strictly diecious forms, as ashes, willows, etc., in which the determiner for maleness is completely suppressed in the sex chromosome bearing the determiner for femaleness, and the female determiner is completely suppressed in the chromosome bearing the factor for maleness.

On this hypothesis we would assume that in the functionally pistillate grape flowers the suppression of maleness has begun and evinces itself in the production of reflexed stamens bearing non-functional pollen, *i. e.*, lacking germ pores (Dorsey, 1913) and containing degenerate generative and vegetative nuclei embedded in apparently normal cytoplasm[2] (Gard, 1913). The period of degeneration of the nuclei is not at all definite. Rarely, the microspore nucleus does not divide. In some cases degeneration takes place directly following the microspore division, in others one nucleus only will degenerate at this time, and in still other cases the two nuclei will appear normal at the time of dehiscence (Dorsey, 1913). Beach (1899), Booth (1902) and Hedrick and Anthony (1915) have shown from pollination and germination tests that occasionally a few pollen grains borne in reflexed stamens are entirely functional. There is an apparent lack of suppression of maleness, occasionally, which allows the development of these normal grains.

Similarly it might be assumed that in the staminate

[2] Pollen of this type should not be confused with abortive pollen which is often produced in hybrids.

flowers suppression of the female determiner has taken place. Booth (1902) and Dorsey (1912) have shown that in practically all staminate grape flowers suppressed pistils are found. In some cases under cultivation and in rare instances in the wild state, the suppression of pistils is less marked and fairly well developed to perfectly developed pistils are formed. On individual plants occasionally all gradations from staminate to functionally hermaphroditic flowers are found.

A third type found under cultivation but which is extremely rare in the wild, is the functional hermaphrodite bearing all hermaphroditic flowers. A discussion regarding the probable origin of this type will be taken up later.

Although breeding work has been carried on for the past twenty-five or thirty years in this country with the grape, apparently little attention has been given to the inheritance of the various flower types, although a knowledge of sex inheritance would be of much value to the breeder. In 1914 Anthony published valuable data on sex inheritance in the grape, but gave no satisfactory interpretation of the results. In 1915 the data again appeared in more detail (Hedrick and Anthony, 1915), and, as no further attempt was made to interpret the results, the writer wishes to present the following as a probable explanation of sex inheritance in the grape, or at least as a working hypothesis for the interpretation of further results which may be obtained.

For the reason that in diecious plants there are apparently definite determiners for maleness as well as femaleness, while in animals it is supposed that males are produced when only one dose of the sex determiner is present, while females are produced if two doses are present, it seems well to use different symbols to designate the sex determiners of plants from those of animals. Therefore, those suggested by Shull (1914, p. 293) in which "the female is assumed to be a neutral homozygote," will be used in the following discussion, namely FF to represent a female and FM to represent a male.

The hermaphrodites would then be designated as $F\widehat{FM}$ or simply as FH. It will be seen from the following discussion that the only formulation which will meet the conditions is that which assumes the female to be a neutral homozygote.

In the above mentioned paper on the "Inheritance of Certain Characters of Grapes" (Hedrick and Anthony, 1915) the authors have concluded that the results obtained on inheritance of sex do not conform to the explanation of sex inheritance dependent on one sex being considered heterozygous and the other homozygous for sex determiners. It appears, however, that by using the hypothesis of partial suppression of sex determiners, the condition in the grape would be in accordance with the assumption of a homozygous condition for femaleness in the functional females, a heterozygous condition for maleness and femaleness in the functionally male plants, and a heterozygous condition for femaleness and hermaphroditeness in some of the hermaphrodites, while others would be homozygous for the hermaphrodite determiners. The authors based their conclusions upon the supposition that the hermaphrodites bearing upright and those bearing reflexed stamens were of a single type genetically, and produced only hermaphrodites and no females when crossed. This assumption seems erroneous.

Using the formulæ suggested above, let us apply them to the data given by the authors, which are as follows:

U × U[3] = 180 U + 47 R	R × R[3] = 16 U + 16 R
U selfed = 673 U + 152 R	R selfed = 94 U + 73 R
U selfed = 18 U + 0 R	
Total.....871 U + 199 R	Total.....110 U + 89 R
Ratio.....4.3 U : 1 R	Ratio.....1.2 U : 1 R

R × U = 207 U + 206 R
Ratio.... 1 U : 1 R
U × R?

Hermaphrodite female × pure male = 56 hermaphrodites + 51 males.

3 "The pollen parent is always placed last." "U" refers to hermaphrodites bearing upright stamens which are usually functional. R refers to hermaphrodites bearing reflexed stamens which rarely produce functional pollen.

In the following discussion the term "female" will refer to the plants bearing morphologically perfect flowers but having reflexed stamens, the pollen of which is not functional. "Hermaphrodite" refers to those bearing perfect flowers, the stamens of which are upright and produce functional pollen. "Male" refers to those plants bearing staminate flowers.

The expectation from the cross "U × U" (hermaphrodite FH × hermaphrodite FH) would be 3 hermaphrodites: 1 female (FF); the hermaphrodites being of two types, viz., 2 FH : 1 HH. This ratio is very closely met in the figures 180 upright and 47 reflexed. "U selfed" should give the same proportions and these are closely approached in the figures 673 upright and 152 reflexed. This assumes the production of homozygous hermaphrodites (HH), which, when either selfed or crossed with other types, produce only hermaphrodites. Apparently the two hermaphrodites which produced 18 hermaphroditic seedlings only, when selfed, are of this genetic constitution. At the Minnesota Experiment Station four hundred seedlings of Beta, open to cross pollination, produced only hermaphroditic flowers; indicating that Beta must be homozygous for the hermaphrodite determiners.[4]

The crosses "R × R" and "R selfed" (female FF × female FF), producing both hermaphrodites and females, might be explained on the hypothesis already given, viz., that of partial suppression of the determiner for maleness in the chromosome bearing the determiner for femaleness.

It has already been pointed out that in the pistillate flowers bearing reflexed stamens, a series of pollen conditions, ranging from those in which the microspore nucleus does not divide, through those in which the generative nucleus aborts directly after the microspore division, to those in which a few normal functional pollen grains are

[4] In *Lychnis dioica* Shull has shown that homozygous hermaphrodites are never produced.

produced, has been found. This very evidently shows that variation in the amount of suppression of the determiner for maleness takes place in the determiner for sex of these normal grains. This normal pollen when used to pollinate pistillate flowers should give, in some cases, females bearing reflexed stamens and in others hermaphrodites, depending upon the extent to which suppression of maleness is lacking in the chromosome bearing the sex determiners of these normal pollen grains.

From the cross "R × U" (female FF × hermaphrodite HF) should be expected females (FF) and hermaphrodites (HF) in the proportion of 1:1. This ratio is met exactly in the cross "R × U" = 207 upright and 206 reflexed.

The following analysis, kindly furnished me by Mr. Anthony of the New York State Agricultural Experiment Station, of the cross "hermaphrodite female × pure male" which produced "56 hermaphrodites + 51 males," shows that both hermaphrodites and females were used as the female parent and that three kinds of males were used, namely, wild males, males one generation from the wild and "intermediates" (males bearing occasionally a few well developed pistils).

	Upright	Reflexed	Males
Hermaphrodite × wild male	7	6	9
Hermaphrodite × male (1 generation from wild)[5]	15	0	14
Female × male (1 generation from wild)[5]	10	3	7
Hermaphrodite × intermediate (origin unknown)	6	4	15
Female × intermediate (origin unknown)	0	1	3
	38	14	48[6]

The various combinations will be considered separately.

The cross hermaphrodite (FH) × wild male (FM) produced 7 hermaphrodites, 6 females and 9 males, somewhat approximating the expected ratio of 1 female (FF) : 1

[5] The result of hermaphrodite × wild male.

[6] These totals do not quite coincide with those given in the published data, as the parents of 7 of the vines were not certainly known and are therefore omitted.

hermaphrodite (FH) : 2 males (FM) and (MH). The male MH is an entirely new genotype but apparently can exist as shown by the next cross, in which a hermaphrodite was pollinated by a male derived from this cross. Fifteen hermaphrodites, no females and 14 males were produced, the expected ratio being (if a male of the type HM were used) 2 hermaphrodites (HF and HH) : 2 males (MH and MF). If a normal male of the constitution FM had been used on a hermaphrodite of the constitution HF we should expect to have produced 1 hermaphrodite (HF) : 1 female (FF) : 2 males (HM and FM). No females were produced. Again we might assume that the hermaphrodite used was of the constitution HH and that a normal FM male was used. In this case we should expect a 1 : 1 ratio of hermaphrodites and males as before, but in this case all of the males would be of the new type HM. It seems, therefore, that the production of this new male genotype (MH) must be admitted. Further evidence for the production of males of this type is produced in the cross female (FF) × male (one generation from wild) which produced 10 hermaphrodites, 3 females and 7 males. If the males used had been of the normal type FM only females and males could have been expected, as are found under wild conditions, and no hermaphrodites. If a male of the type HM were used, however, the expected ratio would be 1 hermaphrodite HF : 1 male FM. The presence of three females, although not expected, from the cross FF × HM can be readily explained, as it has already been pointed out that the males "one generation from the wild" are of the two genotypes FM and HM, but of one phenotype, and therefore could not be distinguished at the time of pollen collection.

The cross hermaphrodite × intermediate (origin unknown) which produced 6 hermaphrodites, 4 females and 15 males, throws some light on the genetic constitution of these intermediates and incidentally upon the suppression of femaleness. Observations on a number of "intermediates" produced at the Minnesota Fruit Breeding

Farm showed that certain clusters of a vine may be entirely staminate, while others of the same vine contain all gradations from staminate to functionally perfect flowers, many of which are capable of setting fruit. There is very evidently a suppression of femaleness in certain parts of these plants and not in others. This raises the question as to whether pollen from the pure staminate clusters can transmit only determiners for maleness and femaleness, or whether they are able to transmit the hermaphrodite condition. Mr. Anthony informs me that the pollen used in the above cross was "most certain to have come from such blossoms" (*i. e.*, from pure male clusters). If the two types of gametes produced by these flowers bear the determiners H and F. respectively, the cross hermaphrodite × intermediate should produce hermaphrodites and females in a 3:1 ratio and no males, while if these male flowers function as normal males and the gametes produced carry the determiners F and M respectively, a ratio of 1 hermaphrodite (HF) : 1 female (FF) : 2 males (HM and FM) would be expected. A close approximation to this ratio was actually produced.

The cross female (FF) × intermediate which produced 1 female and 3 males, gives further evidence that the staminate flowers of the intermediate vines do not produce gametes bearing the hermaphroditic determiner, but act as pure males. Otherwise the appearance of the three males can not be explained.[7] Although the number of vines produced from this cross is small, still the appearance of the three males is extremely significant.

It has already been pointed out that in the wild there are two types of vines, male and female, and that under cultivation a third type, the functional hermaphrodite, is common. We are now in a position to discuss the possible origin of these types. It is clear that both the staminate and functionally pistillate vines carry the determiners for femaleness and maleness, respectively,

[7] Anthony (1914) pointed out the fact that the pollen from these intermediates "seems to behave as the pollen of a pure male."

partially suppressed and therefore, there are two possibilities with regard to the origin of functional hermaphrodites. (1) Maleness may express itself fully in one of the chromosomes bearing the determiner for femaleness in a pistillate plant, and (2) femaleness may express itself fully in the chromosome bearing the male determiner in the staminate plant. I think it can be said definitely that functional hermaphrodites have been developed in both of these ways. The production of hermaphrodites from the cross female × female can hardly be explained on any other basis than entire lack of suppression of maleness in certain gametes bearing the female determiner, while the appearance of well-developed pistils in a few flowers of certain male vines must be the result of lack of suppression of femaleness in at least a portion of the somatic cells of these males.

As there is an apparent segregation in the somatic tissue of these vines, whole clusters and occasionally all clusters on a cane being staminate while others bear many intermediate and perfect flowers, it seems logical to assume that the perfect flowers can transmit the hermaphroditic condition to some of their seedlings through both the male and the female gametes, resulting in either homozygous or heterozygous hermaphrodites, all of whose flowers are perfect.

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ON PRIMARILY UNADAPTIVE VARIANTS[1]

JOHN TREADWELL NICHOLS

American Museum of Natural History

This paper deals with vertebrate *variants* (forms or species of animals more or less related but differing from one another) which, although geographical, are not direct or obvious responses to the environment.

Several types of variants are defined. Representative forms occupying adjacent regions are designated as *adjacent* races or species: Forms intermediate in structure between adjacent forms and occupying territory remote from them as *foreign intermediates:* Related forms occupying the same territory and contrasted in superficial characters as *complements:* Forms separated geographically and showing greater resemblance (not induced by environmental adaptation) than their degree of relationship would presuppose, as *outcrops*.

The hypothesis is advanced that, probably on account of competition, closely related forms are antagonistic.[2] That is, when in touch geographically they tend to force one another apart in superficial characters. If this hypothesis, which seems to fit into certain known facts extremely well, be accepted, it involves a centrifugal force in Evolution opposed to the centripetal tendencies of blood relationship.

It is the main theme of the paper to advance the concept of these two forces as the fundamental framework of evolutionary control, the helm which is swayed by natural selection or other forces.

Larus marinus (the great black-backed gull), which

[1] Paper read before the Section of Biology of the New York Academy of Sciences, February 14, 1916.

[2] See Darwin, "Origin of Species," p. 57. "We can dimly see why the competition should be most severe between allied forms, which fill nearly the same place in the economy of nature."

breeds in the north and reaches our latitude only in winter, finds its nearest relative not in an equatorial species, but in *Larus dominicensis* of the southern hemisphere, below 10° south latitude. This bird is more or less of an intermediate between *Larus marinus* (the great black-back), and *Larus fuscus* (the lesser black-back) which occurs with the former in Europe and is represented by allied forms eastward to the Pacific coast of North America, the mantle of *dominicensis* is very dark as in the larger bird; its size about that of the lesser ones. These are four in number—*Larus fuscus* occurs in Europe east to the Dwina, *Larus affinis* from the Dwina eastward across Asia, *Larus schistisagus* in northern Japan and Bering Sea, *Larus occidentalis* on the west coast of the United States. The four birds of this series are most readily separated by size and proportions of feet. *Affinis* resembles *schistisagus* in the former, *fuscus* in the latter; *schistisagus* resembles *affinis* in the former, *occidentalis* in the latter. That is *affinis* and *schistisagus* are more or less intermediates structurally between the species they separate geographically. For convenience we will call them "adjacent intermediates" as opposed to *dominicensis* which we will call a "foreign intermediate" between *marinus* and birds of *fuscus* group.

A species of vertebrate animals distributed over a wide geographical area often varies in the different regions it inhabits sufficiently to be separable into different intergrading races. Ordinarily no two of these races from the very nature of their origin will be found inhabiting the same region, sometimes they mingle in migration. Often these races are in direct response to different environmental conditions, but sometimes this response can not be traced. Such races form a series comparable to the gulls of the *Larus fuscus* group, and we may call them "adjacent races" and the gulls of the group referred to "adjacent representatives," also we may call *Larus dominicensis* a "foreign representative" of the

black-backs as well as a "foreign intermediate" between the two divisions of that group.[3]

Many of the smaller gulls have a well-defined dark hood in the adult. These hooded species may be divided into two apparently natural groups, the first in which the hood is black, the second in which it is dark brown. In the former group Bonaparte's gull (*Larus philadelphia*) has a great deal of white in the primaries, making a lengthwise band in the wing, conspicuous in life. Bonaparte's gull is found in North America. In the brown-headed *Larus ridibundus* of Europe and Asia the white in the wing makes a similar conspicuous mark, so that no one seeing the two species in life could fail to note the great resemblance. They show distinctly what is ordinarily termed parallelism. Such more or less distantly related, geographically separated parallels, not environmental parallels, are of not infrequent occurrence. For convenience we will call them "outcrops."

Probably the closest relative of *Larus philadelphia* is *Larus saundersi* from the inland waters of China and Mongolia, visiting the coast in winter. This would be a foreign as opposed to an adjacent representative.

Using, for the sake of familiarity, some of the same material we have already considered, if we contrast white-winged *Larus philadelphia*, which is common in the vicinity of New York, with its closest relative common here, the laughing gull *Larus atricilla*, we will find that the two are as different as the limits of the natural black-hooded group of which they are both members will allow. The white primaries of *philadelphia* are contrasted with the unusually dark primaries of *atricilla*, the mantle of the former is pale, that of the latter dark, and there is considerable difference in size. Such contrasted co-existing allies are of frequent occurrence, let us call them "complements."

For my next example I will turn to an entirely different group of animals, the Spanish mackerels, fishes of the

[3] See Matthew, W. D., *Ann. New York Acad. Sci.*, 1915, Vol. XXIV, p. 180, "Principles of Dispersal."

genus *Scomberomorus,* found in warm seas of the world. Along the Atlantic coast of America *S. maculatus* is abundant south to Florida, and *S. regalis* is abundant about Cuba, where *maculatus* is practically unknown. *Regalis* is a close ally, we may reasonably say a derivative of *maculatus,* from which it differs in the scaling of the fins, arrangement of the numerous brownish spots which ornament both species, and in minor characters. Occurring abundantly between the two and associated with one to the north, the other to the south, is unspotted *S. cavalla* (the kingfish of Florida), I will call such an interposed species an "intrusion." Incidentally *cavalla* is a complement of both *maculatus* and of *regalis.*

All the above mentioned cases of variants have this in common: although more or less geographical they are not obvious and direct responses to the environment; probably environment has little to do with them. Apparently we get all the various types of variants as classified where such control is lacking or not essential. It is then pretty certain that variants occur when not induced by environment. Lacking contrary evidence this hypothesis is accepted, and cases where it apparently obtains are alone considered in this article, environmentally induced variants being too common and too generally discussed to need treatment here.

Let us go over the various types of variants, beginning with the simplest, and discuss them in relation to their probable origin. The admittedly inherent tendency to variation in any form would be sufficient, where the form is widely distributed, to break it up into adjacent races. Adjacent representative species are pretty obviously derived from adjacent races. Even temporary isolation so readily explains this slight advance and is so easily assumed, that perhaps we should look no further for explanation. So far we have trodden familiar ground scarcely worthy of mention, and the hypothesis, that namely, probably on account of competition, closely related forms are antagonistic, is conservative enough.

If this hypothesis which seems to fit into the known facts with surprising neatness, be accepted, the writer believes that it will explain a force in direct opposition to the commingling of blood along a line of geographical demarcation of adjacent races, tending to drive those races apart in structure and making possible the derivation from them of adjacent species without geographical isolation. In fact we find in the incompatibility of closely related forms a powerful centrifugal force for differentiating species and forcing them apart[4]—and it also explains complementary coexisting forms, as it would tend to make coexisting forms complementary and would not act to change or eliminate them if they already were so. In the case of the foreign intermediate we have a geographically isolated form less influenced by the centrifugal forces, therefore varying less, retaining intermediate or primitive characters. This centrifugal force should always be considered as balanced against blood-relationship, doubtless the chief cause of resemblance in species. In the outcrop we have a case where the centrifugal force is inoperative and the centripetal tendency brings about a parallelism different in fundamental nature from the more familiar environmentally induced parallels.

There seems to be an analogy between the outcrop and homologous rectigradations in Paleoevolution.

Having given this rather concentrated outline of my hypothesis and the class of facts it is designed to explain it will not be out of place to mention other widely scattered examples of the important classes of variants alluded to. I will begin with the foreign intermediate.

Trichiurus is a long band-like silvery fish with a filamentous tail found along the shores of warm seas, where also occur numerous representatives of the Scombroid or mackerel-like fishes. The two are utterly unlike, yet a clear line of relationship is found through intermediate forms (for instance *Lepidopus*) from the ocean depths.

[4] See, however, Gulick, J. T., "Evolution, Racial and Habitual," 1905, p. 258.

Lepidopus and its confreres are a foreign intermediate family between the mackerels and *Trichiurus,* as *Larus dominicensis* is a foreign intermediate species between the two closely related types of black-backed gull in the north. Please note another analogy. The fundamental philogenic Scombroid characters of *Lepidopus* are a direct response to life at the surface of the mackerel group. Though an intermediate it is impossible (as it is difficult in *Larus dominicensis*) to believe that it occupies the primitive habitat of the group. Other fishes which have persisted in regions distant from the center of ichthyological competition, namely the marine shore line, as in the deep sea or in fresh waters, are in a sense foreign intermediates. Before leaving *Trichiurus* let us glance at its ancestry. The deep-sea *Lepidopus*-like fishes would have little chance in competition with the mackerels in surface waters. They form in a sense a rectigradation (again apologies to Paleontology) development from the mackerels of which *Trichiurus* is the terminal member; but *Trichiurus* is so unmackerel-like that it may and does inhabit the same waters with the mackerels; it is broadly speaking complementary to them.

The tree squirrels are connected with the ground inhabiting spermophiles through the chipmunks (*Eutamias, Tamias* and *Callospermophilus*). Our common eastern chipmunk (*Tamias*) is a boldly marked animal in appearance very much resembling the Rock Squirrel (*Callospermophilus*) of high altitudes of the northwest. The resemblance is more striking than the closeness of blood relationship would presuppose, and *Callospermophilus* may be characterized in this connection as an outcrop. The forms of *Eutamias* inhabiting the same regions as *Callospermophilus* and sometimes found associated with it, are very unlike that animal, and form a very good complement with it. The eastern chipmunk is a foreign intermediate in superficial characters between *Callospermophilus* and western *Eutamias.* Interlocking

of foreign intermediate and outcrop in this case is interesting, it may or may not be significant. Inhabiting more or less the same region as *Callospermophilus,* as do western *Eutamias,* are other spermophiles. They are however speckled or plain colored, little marked for the spermophile group, complements of it, whereas widely separated from it, to the south and east occur spermophiles with sufficient striping to perhaps be considered foreign intermediates between these two northwestern types of spermophile. The above discussion will show how in a single group of mammals, ground squirrels, there exist the various types of variants which have been differentiated above in discussion of fishes and birds.

The most favorable region for fish development and the center of evolutionary competition for fishes is the tropical coral reef. A great many species have the habit of seeking protection among the projections and crevices which there occur in abundance, and these as a rule are very brightly colored, blue, yellow, green, red, or marked with bold bizarre patterns in endless variety. The theory has been advanced and quite generally accepted that the colors harmonized with the brightly colored corals, etc., over which they occurred, but the fact seems to be that although with occasional spots of color the tone of the reef as a whole is comparatively uniform, these bright-colored fishes are very conspicuous swimming over it, in fact give it a good deal of its brilliant appearance. Butterfly fish, wrasses, chromids, parrotfish, etc., belong to this bright, varied crowd. They are safe from all enemies among the labyrinths of the reef and in their evolution have not felt the necessity for concealment.[5] Neutral tones are closer the one to the other than the bright and bizarre, therefore from centrifugal force we should expect, as we find, the bright and bizarre.

Brilliant birds amidst a luxuriant foliage simulate the conditions and the security of reef fishes and frequently

[5] Reighard, J., Public. Carnegie Instit., Washington, 1908, No. 103.

have similarly bright and striking plumage, especially in the males, though the sitting females may be dull. The bright colors of the males may readily have been acquired under control of similar forces. I do not mean to rob sexual selection of its reputed force, but indications are that it is not entirely responsible for all that might be laid to its door. The genus *Dendroica* of small active arboreal birds has developed numerous bright, varied and beautiful colors. Two species perhaps as closely allied as any others are the Blackpoll *Dendroica striata* and Bay-breasted warblers, *Dendroica castanea.* It is interesting to find them very fair complements, the one of the other, in plumage of the breeding male, though females and young are little different; whereas the male blackpoll resembles in color the black and white warbler, *Mniotilta varia,* a distantly related bird of the same family.

Ten years ago the writer had the pleasure of making the acquaintance of the beautiful grey slender-billed fulmar (*Priocella*), on the southern ocean. Sometime afterwards when crossing the North Atlantic he met with the northern fulmar (*Fulmarus*) and was surprised to find the resemblance between the two so great, even carried to a light mark on the wing, very useful in field identifications. Though belonging to the same family the two species are really not very closely allied and are an example of the outcrop. Another case which might be so considered is that of the African true larks or pippits which simulate our meadow larks of the family Icteridæ in color. All passerine birds are so closely allied that the two are not too distantly related to be outcrops, but there are other reasons for thinking that this case is not a very good one, but rather a case of environmental parallelism. The black breast mark, for instance, is so common among ground birds that it probably has concealing value.

The isolated islet of South Trinidad in the south Atlantic is remarkable in that three closely related species

of petrels (Genus *Æstrelata*) are indigenous to it. These are almost identical in size and build but differ markedly in color. *Æstrelata arminjoniana* is bicolored, dark above and white below, *Æstrelata trinitatis* is uniformly dark colored, *Æstrelata chionofara* resembles the former bird, but the white of the underparts spreads up the sides of the neck and on to the back, which is largely white, with dark shafts to the feathers. These birds are obviously very closely related and it is sometimes debated whether or not they are distinct species or merely color phases of one and the same thing.[6] The most convincing evidence, in favor of the hypothesis of distinctness perhaps being that *arminjoniana* and *trinitatis,* forms which have long been known to science, seem to breed on the islet at somewhat different times of the year. *Chionofara* has only recently been described and is so far known from the type specimen only.

The writer has been particularly interested in these birds and has studied them carefully with a view to forming a definite opinion as to their relationship. The bicolored type of plumage represented by *arminjoniana* is perhaps the most common in the cosmopolitan pelagic genus *Æstrelata* of which it is a member, but *arminjoniana* is separated from most of the genus by the greater development of dark color on the side of the neck forming a sort of dark collar. Several uniformly dark colored *Æstrelata* also occur in various parts of the world, comparable to *trinitatis.* The third and recently described form is complementary in color to *trinitatis,* being very white for the genus, and complementary in pattern to *arminjoniana,* having the side of the neck white instead of unusually dark.

If we had three recently evolved forms breeding on the same islet, complementary plumage is what would be expected, and that the three types of *Æstrelata* from there show such plumages is good evidence that they are bona fide forms, not color phases.

[6] Murphy, R. C., *The Auk,* July, 1915, XXXII, No. 3, pp. 342–344.

What color phases are is a matter aside from the thread of the paper but the consideration of this case has led so close to the interesting unsettled problem that the writer hopes to be pardoned for calling attention to certain things about them which he has noticed and which seem to hold good pretty well. First, they are limited to about four manifestations. The timber wolf, *Canis occidentalis* we are told is gray, white, black, or red. The gray squirrel (*Sciurus carolinensis*) is gray or black. The black bear (*Ursus americanus*) black (normal), white (glacier bear) or red (cinnamon bear). The red fox (*Vulpes fulvus*) gray (cross fox), black (silver fox) or red (normal). The screech owl is gray or red.

Students of heredity have shown that the normal gray coat of a wild guinea pig is a composite of black, white and red, which has been broken down by breeding into its constituent parts so that we get black guineas, red guineas, white guineas and guineas with the colors in patches. The colors, you will notice, are the same as those of the color phases occurring in nature and it seems probable that color phases have come about by a similar breaking down and reduction of the normal colors in a species, and have definite limits beyond which they are not likely to go.

In conclusion one might go on indefinitely demonstrating the application of the rough classification of non-adaptive variants proposed in our initial paragraph, limited only by the number of forms one could call to memory. If the significance of the classification has not been exaggerated it is difficult to find another theory which fits as well with the existing phenomena as the one advanced of a centrifugal force, though it is a pure theory and its acceptance a matter of individual taste.

SHORTER ARTICLES AND DISCUSSION

TABLES OF LINKAGE INTENSITIES

In the July, 1916, Naturalist, Professor Emerson gives convenient formulæ for calculating linkage intensities. His formula (I) is especially useful because it is applicable either to cases of coupling or to cases of repulsion. I had independently worked out empirical formulae for calculating coupling and repulsion which are very similar to that given by Emerson; indeed they are identical with it, if 1 is substituted for *r* in cases of coupling and for *s* in cases of repulsion. I had failed to observe, what Emerson shows, that the two formulæ may be given a single generalized form.

TABLE I

The F_2 Ratio, 9 : 3 : 3 : 1, as affected by Coupling or Linkage, A and B entering the F_1 Zygote in the Same Gamete

Ratio, Cross-over to Non-cross-over Gametes	Proportion Cross-over Gametes	F_2 Zygotes				
		AB	Ab	aB	ab	Total
$1{:}x$	$\frac{1}{x+1}$	$3x^2+2(2x+1)$	$2x+1$	$2x+1$	x^2	$(2x+2)^2$
1:1[1]	1/2	9	3	3	1	16
1:2	1/3	22	5	5	4	36
1:3	1/4	41	7	7	9	64
1:4	1/5	66	9	9	16	100
1:5	1/6	97	11	11	25	144
1:6	1/7	134	13	13	36	196
1:7	1/8	177	15	15	49	256
1:8	1/9	226	17	17	64	324
1:9	1/10	281	19	19	81	400
1:99	1/100	29,801	199	199	9,801	40,000
Limiting values[2]		3	0	0	1	4

I had also found it convenient, for my own use, to make out and enter in my notebook tables of equivalent gametic and zygotic series, so that when a suspected case of coupling or repulsion comes to notice the nearest integral gametic series can at once be determined by inspection of the table, without making the calcu-

[1] No coupling.

[2] Not distinguishable from the case in which A and B are due to a single genetic factor.

lation anew. With the idea that these tables may possibly be useful to others, they are given herewith. In making use of such tables it is necessary only to reduce to the basis of a common total the observed F_2 zygotic series and any series of the table with which a comparison is desired. The total given in the table is in each case the lowest one which involves no fractions. If one uses the tables, such formulæ as Emerson's (II–IV) will not be found necessary in estimating the strength of the linkage. Moreover those formulæ are less useful than tables in dealing with the modified dihybrid ratio, 9 : 3 : 4, which happens to have been the first case that I encountered in my own work. The modified ratio as affected by linkage may be read directly from the table by combining classes aB and ab.

TABLE II

THE F_2 RATIO, 9 : 3 : 3 : 1, AS AFFECTED BY REPULSION (NEGATIVE LINKAGE), A AND B ENTERING THE F_1 ZYGOTE IN DIFFERENT GAMETES

Ratio, Cross-over to Non-cross-over Gametes	Proportion Cross-over Gametes	F_2 Zygotes				
		AB	Ab	aB	ab	Total
1:x	$\frac{1}{x+1}$	$2(x^2+2x)+3$	x^2+2x	x^2+2x	1	$(2x+2)^2$
1:1[3]	1/2	9	3	3	1	16
1:2	1/3	19	8	8	1	36
1:3	1/4	33	15	15	1	64
1:4	1/5	51	24	24	1	100
1:5	1/6	73	35	35	1	144
1:6	1/7	99	48	48	1	196
1:7	1/8	129	63	63	1	256
1:8	1/9	163	80	80	1	324
1:9	1/10	201	99	99	1	400
1:99	1/100	20,001	9,999	9,999	1	40,000
Limiting values[4]		2	1	1	0	4

[3] No repulsion.

[4] Not distinguishable from the case in which A and B are allelomorphs.

W. E. CASTLE

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THE AMERICAN NATURALIST

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Devoted to the Advancement of the Biological Sciences with Special Reference to the Factors of Evolution

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THE
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A NOTE ON THE INHERITANCE OF EYE PATTERN IN BEANS AND ITS RELATION TO TYPE OF VINE[1]

FRANK M. SURFACE

In a recent paper (Pearl and Surface, 1915) from this laboratory two varieties of yellow-eyed beans were described and figured under the somewhat provincial names of Improved Yellow Eye and Old-Fashioned Yellow Eye. The type of eye pattern characteristic of each of these varieties is shown below in Figs. 1 and 2. On the Improved Yellow Eye the colored area covers about one fourth the area of the bean. The outer border of the eye pattern is clear-cut and regular, with very little or no spotting on the remainder of the bean.

The Old-Fashioned Yellow Eye pattern (Fig. 2) is much smaller in area and is quite irregular in outline but nevertheless very definite. It consists of at least three color centers: (1) A posterior[2] spot covering the caruncle and extending at least part way around the hilum. Laterally this area is extended into two rather broad wings which reach as far forward as the micropyle. (2) An anterior spot surrounding the micropyle, and (3) an anterior stripe which may or may not connect with the micropyle spot.

In connection with other work a number of crosses have

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 99.

[2] The *posterior* end of a bean is that end of the hilum at which the caruncle lies. It is the end opposite the micropyle.

been made between these two varieties. Something over 40 cross-pollinated beans have been secured. Of these, 15 have been grown at least as far as the F_1 generation. These 15 plants gave a total of 295 F_1 beans. Except for some minor fluctuations these F_1 beans were all alike, but differed markedly from either parent. In the notes these F_1 beans have been designated "Piebald" because of the very irregular spotted pattern. Fig 3 shows a typical piebald pattern. In addition to the spotting these beans differ from the Improved Yellow Eye in having a very irregular outline to the colored area. While the pattern is somewhat variable, there is never any difficulty in distinguishing this from the typical Improved Yellow Eye pattern.

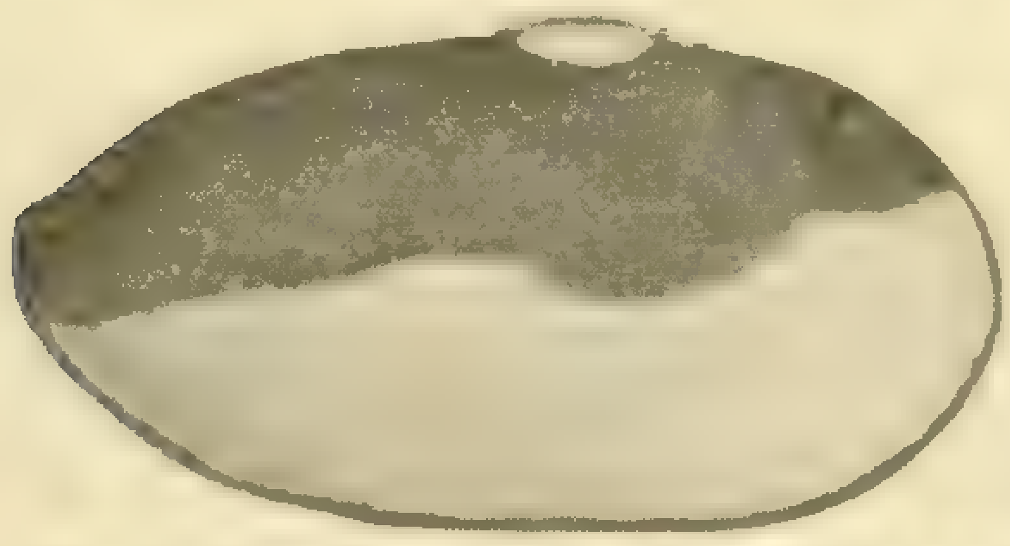

FIG. 1. Typical Improved Yellow Eye color pattern.

FIG. 2. Typical Old-Fashioned Yellow Eye color pattern.

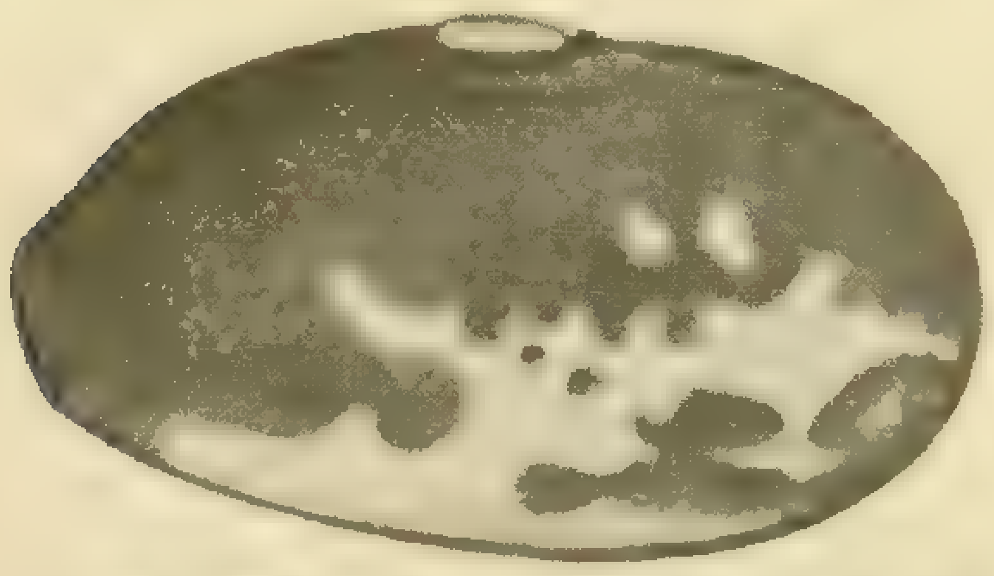

FIG. 3. Typical "piebald" color pattern occurring on the F_1 beans of a cross between the Improved and Old-Fashioned Yellow Eye types.

Up to the present time only a few of these hybrids have been carried to the F_2 generation. However, enough have been obtained to show that these piebald beans give both parent types and also more beans with the piebald pattern. It is very probable that only these three types occur in the F_2 and later generations.

While the data so far obtained from hand-pollinated hybrids are not sufficiently extensive to warrant further discussion, certain other data have been obtained which have a bearing on this subject.

In 1911 and 1912 the Experiment Station grew a number of plots planted with different strains and varieties of beans. Among these were a number of strains of Improved and Old Fashioned Yellow Eye. In some cases plots of these two varieties were located near each other. Seed from some of the 1911 plots were planted in 1912.

A considerable number of plants in these plots showed that the seed had been cross-pollinated by bumble-bees the year before. Among the plants in the Yellow Eye plots there were a number which bore typical piebald beans similar to that shown in Fig. 3. Some of these piebald plants were harvested separately and their progeny continued in a small way inside a screened cage. By the spring of 1915 it had been ascertained that a cross between an Improved and an Old-Fashioned Yellow Eye resulted in such a piebald pattern. Accordingly a considerable number of these piebald beans were grown in 1915. The following paper is based upon the data from these natural hybrids.

Table I gives the detailed data relative to the offspring

TABLE I

Showing the Segregation in the Progeny of Piebald Beans

Pedigree No.	Year	Row	Piebald	I. Y. E.	O. F. Y. E.
1294–5	1912	30	11	3	4
	1913	87	—	3	2
		88	5	—	—
	1914	31	4	—	—
	1915	58	2	3	4
		269	12	7	3
		270	7	2	5
		271	8	1	3
		272	10	3	1
		273	6	—	2
		274	6	3	6
Total for 1294–5			*71*	*25*	*30*
1311	1913	104	3	—	—
	1914	32	2	1	2
	1915	292	13	7	4
		295	10	4	5
		296	6	1	3
		297	3	2	3
Total for 1311			*37*	*15*	*17*
153 X	1915	303	5	3	4
		304	8	1	3
Total for 153 X			*13*	*4*	*7*
1318	1915	307	5	2	5
		308	5	1	4
Total for 1318			*10*	*3*	*9*
1321	1915	310	8	3	3
		311	7	3	4
Total for 1321			*15*	*6*	*7*
Grand total			146	53	70

of 269 piebald beans. It will be understood that each row was planted from the offspring of a single plant. Not all of these beans can be considered as belonging to the F_2 generation. A portion of these certainly belong to the F_3 and later generations. This question will be considered further in a later paragraph.

From this table it will be seen that only three kinds of beans were obtained from these piebald seed. These were piebald, Improved Yellow Eye and Old-Fashioned Yellow Eye. This fact, in connection with the evidence obtained from controlled pollinations as noted above, makes it practically certain that these piebald beans are hybrids between these two varieties of Yellow Eye beans.

Further, with the exception of three small rows none of of these piebald beans gave evidence of breeding true. In each of these three cases some of the piebald beans have split in later generations. Thus in pedigree No. 1294–5 the 1914 Row 31 is the offspring of one of the five piebald plants in the 1913 Row 88. It seemed possible that this line was breeding true. However, the 1915 Row 58 is the offspring of a plant from Row 31 of the year before, and Row 58 gave all three types, so that both of the preceding rows must have been heterozygous. If larger numbers had been grown from the same seed they would undoubtedly have thrown all three types.

The evidence thus indicates that the piebald pattern is the expression of the heterozygous condition of the factorial difference between these two types of Yellow Eye beans. A similar conclusion was reached by von Tschermak (1912). He obtained spotted beans very similar to our "piebald" from crosses between eyed and white or eyed and solid color beans. These piebald beans were always heterozygous, throwing on the one hand a large eye with regular outline corresponding with our Improved Yellow Eye and on the other hand a small-eyed bean. Judging from his figures (p. 208) von Tschermak's small-eyed bean had nothing corresponding to the peculiar pattern on our Old-Fashioned Yellow Eye. However, in

relative quantity of pigment these beans agree very well.

Von Tschermak assumed a unifactorial difference between the large and small-eyed beans, with the spotted pattern as the heterozygote. In the F_2 generation he obtained a 1:2:1 ratio.

Returning now to our own data as given in Table I it is clear that if the difference between the Improved and Old-Fashioned patterns is due to a single factor we should expect in the segregating generations 2 piebald:1 I. Y. E. :1 O. F. Y. E. The numbers obtained in Table I will hardly support this view. 146:53:70 can hardly be looked upon as a 2:1:1 ratio. It is true that the deviation is not so great, but that these observed numbers might be chance fluctuations from a 2:1:1 ratio. On the theory of probability the odds against the occurrence of such a deviation are about 5 to 1.

Of the more common Mendelian ratios the observed figures are much more closely fitted by 9:3:4. The observed and expected numbers in this case are

	Piebald	I. Y. E.	O. F. Y. E.
Observed No.	146	53	70
Expected No. on 9:3:4 ratio	151.3	50.4	67.3

It is clear that there is a very reasonable agreement.

Further evidence in support of the view that the segregation is not 2:1:1 is found by examining Table I in more detail. Thus the totals for each of the five pedigrees show an excess of Old-Fashioned Yellow Eyes over the Improved type. In three of these pedigrees the number of plants is relatively small. However, the cumulative evidence makes it almost certain that the deviations are not due to chance.

It was stated above that only a portion of these plants belonged to the F_2 generation. In a bifactorial character considerable difference might be introduced by the combination of data from different generations. From the records it is known that all the plants in pedigree Nos. 153 X, 1318 and 1321, together with two rows, 104 and 292,

from pedigree 1311, belong to the F_2 generation. Taking these plants alone, we have the data given in Table II.

TABLE II

SHOWING THE SEGREGATION IN THE F_2 GENERATION FROM PIEBALD BEANS

Piebald	I. Y. E.	O. F. Y. E.
54	20	27

It is seen at once that there is again the same relative excess of O. F. Y. E. over I. Y. E. that is shown by the complete data in Table I. The expectation on the 2:1:1 ratio is 50.5:25.3:25.3, while on the 9:3:4 ratio the expectation is 56.8:18.9:25.3. It will be seen that the latter figures more nearly fit the observed numbers.

A 9:3:4 ratio presumes a bifactorial composition. However, a moment's consideration shows that such a ratio cannot have its usual significance in this case. If this were the usual bifactorial segregation, one out of every nine F_2 piebald beans ought to breed true in the third generation. Yet out of 15 rows from piebald beans which certainly belong to the F_3 or F_4 generation not a single one bred true.

Further, one half of the F_2 Old-Fashioned Yellow Eye segregates and two thirds of the F_2 Improved Yellow Eye segregates ought to show segregation in the third generation. In 1915, 43 Old-Fashioned Yellow Eye, F_3 plants were grown and every one bred true. At the same time 38 F_3 plants were grown from Improved Yellow Eye seed. Thirty-seven of these gave typical Improved Yellow Eye beans, but one plant gave piebald beans. The F_2 plant which furnished this latter seed was grown without any protection from insects in 1912 and it is very probable that the one I. Y. E. bean which gave piebald seed was due to insect pollination with Old-Fashioned Yellow Eye pollen. This is all the more probable because the ratio 1:37 is by no means what would be expected on the usual bifactorial hypothesis.

The evidence is fairly conclusive that the I. Y. E. and the O. F. Y. E. segregates breed true and that beans with

the piebald pattern are always heterozygous. These results could be very simply interpreted on a single-factor hypothesis, but the numerical results do not fit the 2:1:1 ratio demanded by that hypothesis.

While the data at hand are not as extensive as one might desire in order to build a complete theory, yet there is much to be said in favor of the following provisional hypothesis. Let *I* be a factor which in its homozygous condition *II* produces the Improved Yellow Eye pattern. Then *Ii* will be the zygotic constitution of the piebald plants and *ii* that of the Old-Fashioned Yellow Eye pattern. Assume further a lethal factor *L* independent in its segregation and of such a nature that *LL* in the presence of *II* produces a non-viable zygote. The complete F_2 segregation would then be as follows:

1	*I I L L*	Non-viable[3]
2	*I I L l*	I. Y. E.
1	*I I l l*	
2	*I i L L*	
4	*I i L l*	Piebald
2	*I i l l*	
1	*i i L L*	
2	*i i L l*	O. F. Y. E.
1	*i i l l*	

Such a segregation would result in the ratio 8 piebald: 3 I. Y. E. : 4 O. F. Y. E. Testing this ratio against the total observed numbers in Table I we get

	Piebald	I. Y. E.	O. F. Y. E.
Observed No.	146	58	70
Expected No. on 8 : 3 : 4 ratio	143.5	53.8	71.7

It is seen that there is a very close agreement between the observed and expected numbers: much closer, in fact, than in the case of the 9:3:4 ratio previously used.

[3] The same result would be obtained if *ll* in the presence *II* produced a non-viable zygote. This point could be determined by suitable crosses between the F_2 segregates.

In the case of known F_2 plants, as given in Table 2, the results are

	Piebald	I. Y. E.	O. F. Y. E.
Observed No.	54	20	27
Expected No. on 8 : 3 : 4 ratio	53.8	20.2	26.9

Here again there is a very remarkable agreement. In fact all of the data at hand fit into this theory very nicely. Final proof of its correctness or incorrectness can only come with more extended crossings between the segregates and with the parent stocks. Such experiments are now under way.

Relation of Eye Patterns to Type of Vine

Two years ago while going over some data from pure lines of Yellow Eye beans grown inside a screened enclosure the writer was struck by the fact that with few exceptions all of the O. F. Y. E. pure lines had the bush type of vine, while nearly all the I. Y. E. lines were classed as short runners. This point was further emphasized by the observation that in several cases the segregation from piebald beans showed that all of the O. F. Y. E. segregates were bush beans and all the I. Y. E. were runners. It was, therefore, of some interest to tabulate the data relative to type of vine in connection with the eye pattern.

The classification of plants with reference to type of vine has usually been made at the time of harvest. In some years the plants grown inside the screened cage have been classified as to vine type shortly before harvest. In either case the plants were mature or practically so. The plants grouped under the term "bush" are those which show determinate growth, terminal inflorescence, and lack the ability to twine about supports. The "runner" plants show axillary inflorescence and the twining habit (circumnutation). All of the runner beans considered in this paper are of the short runner or short pole type, rarely reaching a total height of more than 125 cen-

timeters. Usually they develop few branches. Under ordinary conditions such beans do not show indeterminate growth. However, from the investigations of Emerson (1916) it is probable that they would do so if growth were not stopped by unfavorable conditions or excessive seed production.

Data as to type of vine are available from 247 of the plants given in Table I. Table III shows the distribution of the type of vine for each of the three eye patterns. The data for each pedigree number are summarized separately.

TABLE III

DISTRIBUTION OF TYPE OF VINE FOR EACH OF THE THREE EYE PATTERNS

Pedigree No.	Piebald		I. Y. E.		O. F. Y. E.	
	Runner	Bush	Runner	Bush	Runner	Bush
1294–5	40	19	12	6	0	27
1311	18	19	4	11	0	17
153 X	3	10	2	2	0	7
1318	0	10	0	3	0	9
1321	9	6	6	0	0	7
Total	70	64	24	22	0	67

The most striking thing in connection with this table is the complete absence of runner vines among the Old-Fashioned Yellow Eye beans. Apparently the gene for bush type of vine is closely associated with the gene for the Old-Fashioned Yellow Eye pattern. That this association is not absolute under all conditions is indicated by the fact that I now have two strains of Old-Fashioned Yellow Beans of unknown origin which for several generations have bred true to a distinct runner type of vine. A number of crosses have been made using these runner types of Old-Fashioned Yellow Eye. It is hoped that these and other experiments which have been started will throw some light upon this question.

Attention may be called to the apparent 1:1 ratio of runner to bush in the case of the piebald and Improved Yellow Eye beans. Emerson (1904, 1916), von Tschermak (1904, 1912) and others have shown that in crosses between tall (runner) and dwarf beans the expected F_2

ratio is 3 tall to 1 dwarf. Obviously the present data are of little use in the study of this question because in the first place it consists of a mixture of F_2, F_3 and F_4 plants and in the second place the vine characters of the parents in the different crosses are unknown. It is quite possible that the parents in the case of pedigree No. 1318 were both of the bush type. The 22 plants in the F_2 generation in this strain are all of the bush type.

The only reason for presenting the data in Table III at this time is to call attention to the relation between the bush type of vine and the Old-Fashioned Yellow Eye pattern. There seems to be no question but that these two characters are closely associated.

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CHROMOSOME STUDIES ON THE DIPTERA

III. Additional Types of Chromosome Groups in the Drosophilidæ

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In connection with other work on the Drosophilas I have for some time been engaged in a comparative study of their chromosomes, with especial reference to possible phylogenetic relationships between different species. A short preliminary report of this study was published two years ago (Metz, '14) after five types of chromosome groups had been found among eleven species. More recently I have studied fifteen additional species of *Drosophila,* one of *Cladochæta* and two of *Scaptomyza* (related genera), and have found several more types of chromosome groups. Altogether twelve main types and several sub-types have been identified—a series more extensive, I believe, than any heretofore recorded among allied species. Of these twelve types all but one are represented in the genus *Drosophila.*

The study has not yet advanced far enough to fulfil the purpose for which it was originally undertaken, but in view of the widespread interest recently attracted to the Drosophilas as objects of genetic research it seems desirable briefly to describe the chromosomes of the species thus far examined without awaiting the completion of the original investigation. In doing this I shall endeavor merely to give an accurate presentation of the chromosomal data, without dwelling on the theoretical considerations they may suggest, considerations which can receive adequate treatment only after many more species have been examined.

Since in almost every case larval or pupal stages are

the only ones suitable for study, it has been necessary to breed the various species in confinement in order to determine their chromosome groups. As a result only about half of those collected have been studied cytologically. Other determinations will be reported in the future as they are obtained.

The material has been secured from four regions, New York, Massachusetts, Alabama and Cuba, with the exception of one species (not found in these localities) from California and Oregon.[1] Fourteen of the twenty-nine species are undescribed and are here given the manuscript names of Dr. A. H. Sturtevant.[2] Descriptions of them are in press.

Most of the chromosome descriptions in the present paper are taken from pedigree material, either first or second generation from wild flies, and the results have been checked up in such a way as to make it very improbable that serious errors have crept in. As mentioned in previous papers the chromosomes of these flies stand out with diagrammatic clearness when favorable figures are secured; and since they are uniformly arranged in pairs and are often of various sizes they offer admirable material for a comparative study. This makes it possible to classify the members of each chromosome group according to their size and shape, and to assort the groups into definite types according to their configuration.

Description of Types

The terminology used in describing the chromosomes will be the same as that used in my previous paper ('14).

[1] The collections, of course, include only a fraction of the existing species within these areas, to say nothing of those in surrounding regions. For more detailed locality references see description of types.

[2] This investigation would have been practically impossible without the cooperation of many friends. In addition to Dr. Sturtevant, to whom I owe all of the identifications, and cultures of several species, I am under obligation to Professor F. S. Earle, Dr. Carlos de la Torre and Mr. C. T. Ramsden for many courtesies shown to me while collecting in Cuba, and to Messrs, L. L. Gardner and G. F. Sykes for Pacific coast material, including *D. obscura.*

Certain recurring kinds will be distinguished in advance and will be designated by name or letter in the specific descriptions. These are as follows:

V. Long, V-, U-, or dumb-bell shaped chromosomes, attached to the spindle at the median constricted portion (apex of the *V*).

r. Rod-like, straight chromosomes, approximately half the length of *V*, attached to the spindle at one end, and radially arranged in metaphase.

c. Short, curved chromosomes, differing from *r* only in form and (probably) in having a median attachment to the spindle.

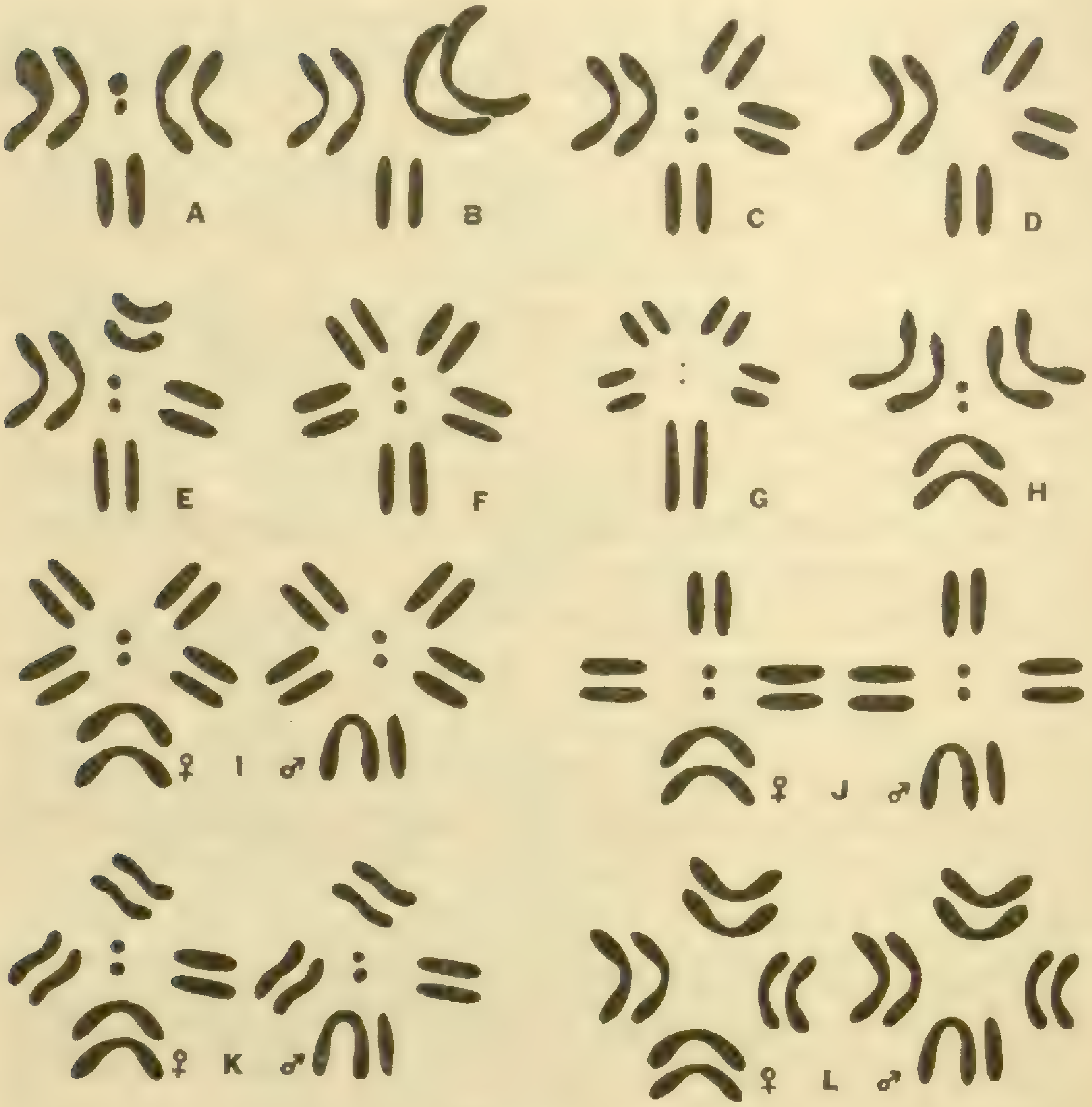
m.[3] Minute, spherical or slightly elongate chromosomes, usually located in the center of the metaphase plate.

To these symbols must be added *XX* and *XY*, used to designate the sex chromosomes wherever they have been definitely identified. In some species they are straight, in others V-shaped. Identification of the sex chromosomes and determination of the *XY* relations in the males has been one of the most difficult features of the study, owing to the extreme scarcity of spermatogonial and spermatocyte figures; but it has been made in as many cases as possible and has been of great usefulness in comparing different groups.

The order or sequence in which the types are described is a purely arbitrary one, and is not intended to indicate any genetic relationship.

In order to avoid duplication of figures chromosome groups which have previously been described and figured are not reproduced here unless they are of especial interest. The series of types as a whole may best be understood from an examination of diagrams *A* to *L*, which represent schematically, but accurately, the twelve main types.

[3] The term *m*-chromosome, borrowed from Wilson ('05), is used here in a purely descriptive sense, and is not intended to signify any relationship with the *m*-chromosomes of the Hemiptera.



Type A

Represented by

Drosophila ampelophila Loew. Cosmopolitan.[4] (See Stevens, '08, Figs. 57–60, 80–82, Metz, '14, Figs. 4 and 5, Bridges, '16, Figs. 1–4, Metz, '16, Fig. 19.)

Drosophila amoena Loew. New York. (Metz, '14, Figs. 1–3, Metz, '16, Figs. 13–16.)

Drosophila busckii Coq. New York. (Metz, '16, Figs. 17 and 18.)

Drosophila bromeliae Sturtevant mss. Cuba. (Fig. 1.)

Drosophila dimidiata Loew. Alabama. (Metz, '16, Fig. 20.)

[4] With the exception of this species, which has been studied by several investigators, localities cited are those in which my cytological material has been obtained.

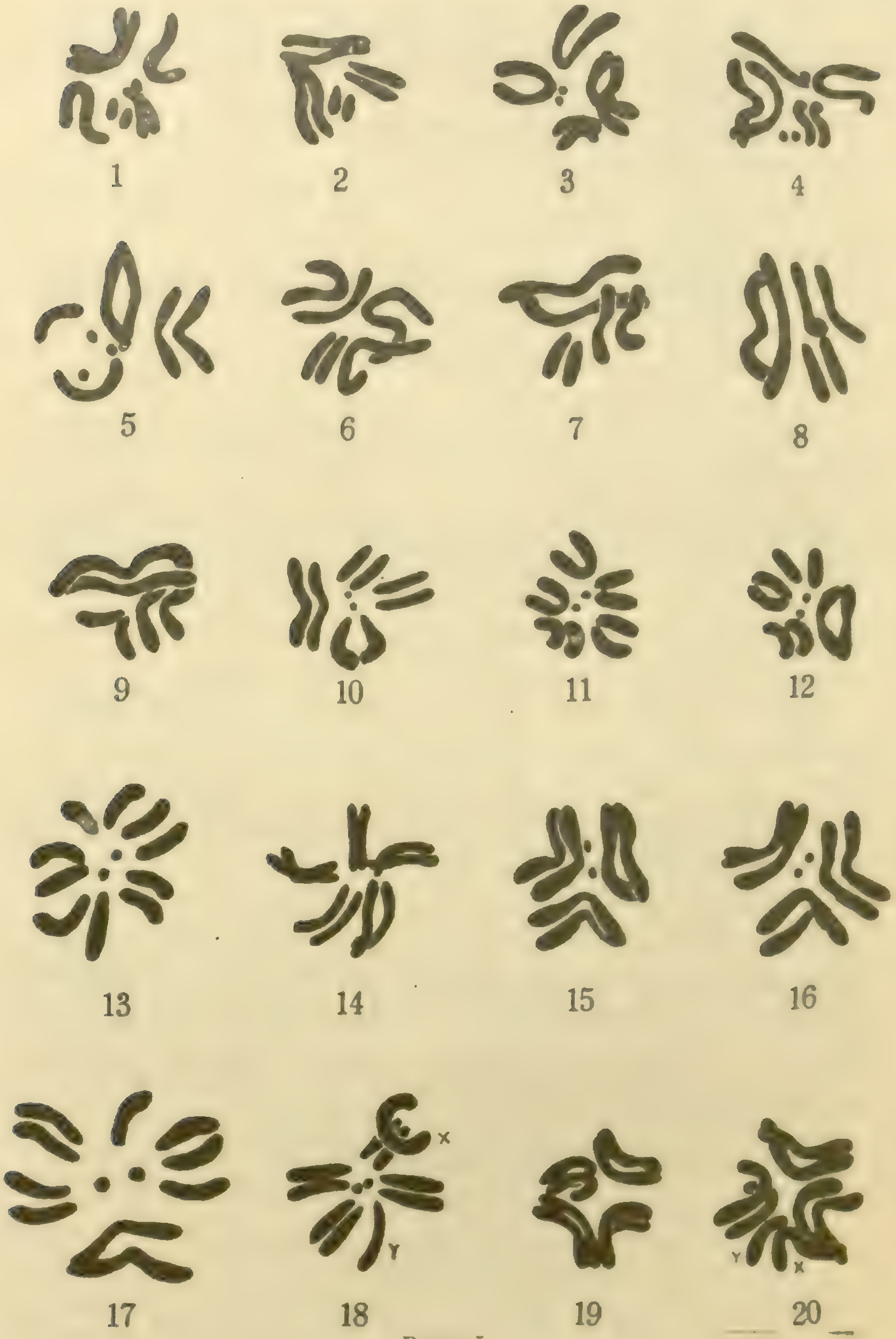


PLATE I

Explanation of Figures

All figures were drawn with the aid of a camera lucida, using Zeiss 1.5 mm. apochromatic objective and compensating ocular number 12, with tube length of 160 mm. The drawings are reproduced natural size. With the exception of number 17 all are taken from sections cut 5 microns thick.

Drosophila floræ Sturtevant mss. Cuba. (Fig. 2.)
Drosophila limbata Will. Cuba. (Metz, '16, Figs. 10–12.
Drosophila procnemis Will. Cuba. (Metz, '16, Figs. 34–36.)
Drosophila quinaria Loew. New York. (Metz, '14, Fig. 7.)
Drosophila robusta Sturtevant mss. New York. (Figs. 3 and 4; Metz, '16, Figs. 7–9.)
Drosophila saltans Sturtevant mss. Cuba.
Drosophila pallida Will. Cuba.
Scaptomyza graminum Fall. New York. (Fig. 5; Metz, '16, Figs. 4–6.)

This is type I of my previous paper, and is the prevailing type among the species studied, being represented by thirteen of the total twenty-nine. It consists of two pairs of long, V-shaped chromosomes, one pair of straight sex chromosomes, and one pair of *m*-chromosomes.

Slight modifications of this type are found in certain species, usually characterized by peculiarities in the sex chromosome pair. In *D. ampelophila* it appears from the work of Bridges ('16) that the *Y*-chromosome, instead of being straight as is the *X*-chromosome, is hook-shaped or V-shaped, although never large enough to be confused with the V-shaped euchromosomes. My observations confirm those of Bridges in this regard with the exception of two figures in which *X* and *Y* appear equal and straight. Perhaps these are due to accident, but they are entire figures and normal in other respects.

FIG. 1. *Drosophila bromeliæ* Sturtevant mss., spermatogonium.
FIG. 2. *Drosophila floræ* Stt. mss., ovarian cell.
FIGS. 3 and 4. *Drosophila robusta* Stt. mss., spermatogonia.
FIG. 5. *Scaptomyza graminum*, spermatogonium.
FIGS. 6–9. *Drosophila carlei* Stt. mss., ovarian cells.
FIG. 10. *Scaptomyza adusta*, ovarian cell.
FIGS. 11 and 12. *Drosophila neglecta* Stt. mss., ovarian cells.
FIG. 13. *Drosophila similis*, ovarian cell.
FIG. 14. *Drosophila cardini* Stt. mss., ovarian cell. The *m*-chromosomes are not visible in this figure, but are evident in other cells of the same ovary.
FIGS. 15 and 16. *Cladochæta nebulosa*, ovarian cells.
FIG. 17. *Drosophila repleta*, variety *b*. ovarian cell. Taken from an aceto-carmine smear preparation.
FIG 18. Same, spermatogonium, from a section.
FIG. 19. *Drosophila caribea* Stt. mss., ovarian cell.
FIG. 20. Same, spermatogonium.

In *D. amoena* and *S. graminum* spermatogonial figures indicate that *X* and *Y* are unequal and straight (Fig. 5, and Metz, '14, Figs. 2 and 3); while in *D. buschii, D. floræ, D. bromeliæ, D. robusta* and *D. limbata* no inequality is evident. The other species have not been examined with respect to spermatogonial groups. In *D. robusta* the rod-like members (sex chromosomes?) appear to be hook-shaped and to be attached sub-terminally to the spindle in much the same manner as the *Y*-chromosome in *ampelophila*.

Another modification or sub-type is represented by *D. floræ* and *D. bromeliæ*, in which the *m*-chromosomes are materially larger than in the other species. Indeed, those of the former are so large as to suggest a transition between *m*-chromosomes and *r*-chromosomes.

Type B

Represented by

Drosophila earlei Sturtevant mss. Cuba. (Figs. 6–9.)

This interesting type consists of one short rod-like pair and two large V-shaped pairs, one of which is much longer than the other. No trace of *m*-chromosomes has thus far been found in the fifteen or twenty figures I have studied. Unfortunately I have not yet secured good spermatogonial figures and am unable to identify the sex chromosomes.

In many respects type *B* is of greater interest than any other type of chromosome group I have studied, for it not only contains the smallest number of chromosomes thus far found among the higher flies,[5] but each of its three pairs is conspicuously different from either of the other two, making possible an individual identification of the chromosomes not obtainable, with such a degree of certainty, in any other known species of *Diptera*. If a genetic continuity of chromosomes be admitted there can be no question that here each paternal chromosome as-

[5] Two species of Culicidæ (*Anopheles punctipennis* and *Culex pipiens*) also have three pairs of chromosomes. See Stevens, '11.

sociates with its corresponding maternal mate. (See Metz, '16, p. 251.)

Type C

Represented by

Drosophila ornatipennis Will. Cuba. (Metz, '16, Fig. 21.)

Scaptomyza adusta Loew. New York. (Fig. 10; Metz, '16, Fig. 22.)

Type *C* corresponds to type IV of my previous paper ('14), but the single species formerly referred to it has been transferred to type *E*. Chromosome groups of type *C* are composed of one large V-shaped pair, one long, straight sex chromosome pair, two shorter rod-like pairs and one small *m*-pair. In both species spermatogonial figures show a noticeable inequality between *X* and *Y*; thus identifying the sex chromosomes.

Type D

Represented by

Drosophila tripunctata Loew. New York. (Metz, '14, Figs. 21–26.)

Type *D* corresponds to type *V* of my previous paper ('14) and includes only one species. It differs from type *C* in lacking the *m*-chromosome pair, and in sex chromosome relations. *X* and *Y* are apparently equal in size and similar to the rod-like euchromosomes. Their identification is based solely upon their precocious contraction in prophase (see Metz, '14, p. 52), and hence may be held with some reserve, but from analogy with species of types *A* and *C* it seems highly probable that the identification is correct.

Many preparations have been made from material of this species in an attempt to discover an *m*-chromosome pair such as is found in most other species of *Drosophila*, but although stocks have been obtained from several localities and various methods of fixation have been used no trace of the pair has been observed.

Type E

Represented by

Drosophila melanica Sturtevant mss. (two varieties). New York, Alabama. (Figs. 11, 12; Metz, '16, Figs. 23–26.)

The chromosomes of this species resemble those of type *C*, with the exception of one of the short pairs, which is curved or U-shaped instead of straight. Such a difference in shape is apparently associated with a different mode of attachment to the spindle, and seems to be a characteristic feature. In my previous paper ('14) *D. melanica* was cited as an example of the type corresponding to *C* of the present paper, and the curved shape of this pair was not considered significant; but more recently I have examined many additional figures and have become convinced that the character is normal and sufficient to distinguish the two types. The few spermatogonial figures I have examined closely resemble those of female groups and give no evidence of an unequal *XY* pair.

The two varieties[6] of *D. melanica*, although refusing to hybridize, are very similar in external appearance and indistinguishable in chromosomal characters.

Type F

Represented by

Drosophila virilis Sturtevant mss. New York City. (Metz, '14, Figs. 11–13, Metz, '16, Fig. 2.)

Drosophila similis Will. Cuba. (Fig. 13.)

Drosophila ramsdeni Sturtevant mss. Cuba. (Metz, '14, Fig. 10, Metz, '16, Fig. 3.)

Drosophila cardini Sturtevant mss. Cuba. (Fig. 14.)

Drosophila modesta Sturtevant mss. Alabama.

Drosophila repleta Woll., variety *a*.[7] Cuba, Texas. (Metz, '14, Figs. 8 and 9.)

This type (Type II of the previous paper) differs from *C* in having two pairs of rod-like chromosomes in place of the large V-shaped pair, and from type *A* in possessing

[6] For discussion of these see forthcoming paper by Dr. A. H. Sturtevant.

[7] See also type *I*.

four rod-like pairs in place of two V-pairs. Next to type *A* this type is of most frequent occurrence, being represented by six of the twenty-nine species.

Spermatogonial figures have been examined in only one of these species, *D. virilis,* and here no conspicuous inequality between *X* and *Y* is to be seen. One pair of chromosomes appears to be larger in nearly all figures of either sex, and a slight difference in length between the two members of this pair may be seen in some male figures, but it may be purely accidental.

Type G

Represented by

Drosophila funebris Fabr. New York, California, North Dakota. (Metz, '14, Figs. 14–17,[8] Metz, '16, Figs. 27–33.)

This interesting type (type III of the previous paper) is apparently a modification of type *F,* but differs from it in the relative proportions of all of the chromosomes. The *m*-chromosomes are so minute in most specimens as scarcely to be visible, and for this reason were entirely overlooked at first. Their conspicuousness doubtless varies with the amount and kind of stain, and with the fixative used, but even after making full allowance for this there can be no doubt that the pair is much smaller here than in most other species. Otherwise the type is characterized by the smaller size of the short, rod-like chromosomes and the greater length of the longest (sex chromosome?) pair. As in the preceding case no conspicuously unequal *XY* pair is to be found in the males, although a noticeable difference between the two large chromosomes may be seen in some of the figures.

Type H

Represented by

Cladochaeta nebulosa Coq. Cuba. (Figs. 15 and 16.)

This species—the only known member of the genus—is

[8] Fig. 14 (Metz, '14) and Fig. 27 (Metz, '16) are from the same cell; the latter drawn after the *m* chromosomes were discovered.

included in the present review because of its obvious relationship to the true Drosophilas. The type of chromosome group which it represents is the only one of the twelve not thus far found in some species of *Drosophila*, and its general similarity to some of the *Drosophila* types is marked. Female groups consist of three similar pairs of long, V-shaped chromosomes and one small pair of *m*-chromosomes. Unfortunately the species breeds very poorly in confinement and no male preparations were secured. It is almost certain, however, that one of the long pairs is the sex chromosome pair.

Type I

Represented by

Drosophila repleta Woll., variety *b*. New York, Massachusetts, California. (Figs. 17 and 18; Metz, '16, Figs. 39–41.)

In my 1914 paper *D. repleta* was referred to the type corresponding to *F* of the present study, but it is now evident that two very similar but distinct varieties of the species occur, characterized, among other things, by decidedly different sex chromosomes. In one, the sex chromosomes are short and rod-like in the female and presumably so in the male, while in the other they are long and V-shaped in the female and markedly unequal in the male. The latter represents the present type *I*. The difference between the two may be readily appreciated by an examination of diagrams *F* and *I*. Although it relates only to the sex chromosomes it is very striking in the females, and easily separates the two varieties into distinct types. The fact that the two forms can not be induced to hybridize lends support to the chromosomal evidence of their distinctness, but externally they are astonishingly similar and are referred to the same species by Sturtevant.[9]

[9] It may be noted that these are not the "light and dark" varieties described by Sturtevant ('15), both of which belong to type I.

Type J

Represented by

Drosophila obscura Fall. California and Oregon. (Metz, '16, Figs. 44–50.)

Ovarian cells of *D. obscura* contain three rod-like euchromosome pairs, one small *m*-chromosome pair and one very long, V-shaped sex chromosome pair (diagram *J*, ♀). In the male the sex chromosomes are very dissimilar, *Y* being straight and only about half as long as *X*.

Type K

Represented by

Drosophila affinis Sturtevant mss. Alabama. (Metz, '16, Figs. 42 and 43.)

In general this type resembles the last, but differs in having two S-, or hook-shaped pairs in place of rod-like ones. Apparently this peculiar shape is due to a subterminal attachment to the spindle, although I have been unable to get figures actually demonstrating the attachment. In some cases one or both pairs extend radially from the center of the figure as if they were attached terminally, but usually their position is characteristically that described above. In any event the two pairs are readily distinguished from any others of the group, unlike those of *D. obscura*.

Type L

Represented by

Drosophila caribea Sturtevant mss. Cuba, Panama. (Figs. 19 and 20.)

This type is radically different from any of those described above, and like the two preceding is represented by only one species. Female (ovarian) groups are composed of four long V-shaped pairs of chromosomes, one, of which is shorter than the other three. In the male one pair is conspicuously unequal, much as in types *I*, *J* and *K*, but I have been unable to determine with certainty whether this is the small pair or one of the large ones. It is represented as a large one in the diagram (*L*, ♂).

Several of the species contained in this survey have been, or are being used in genetical studies. With the exception of the well-known *D. ampelophila* these are included in the following list, together with references to literature dealing with them, so far as known to me:

D. repleta, type *H.*, Sturtevant, '15, Hyde, '15.

D. affinis, type *J.* Hyde, '15.

D. tripunctata, type *D.* Metz and Metz, '15.

D. virilis, type *F.* Metz and Metz, '15.

D. similis, type *F.* In press.

D. obscura, type *I.* In press.

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THE SHAPE OF THE STERNUM IN SCORPIONS AS A SYSTEMATIC AND A PHYLOGENETIC CHARACTER

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It is generally recognized that the shape of the sternum furnishes one of the important characters for the distinction of families in recent scorpions. The small family Bothriuridæ is the only one in which the sternum is composed of two transverse plates. This family includes seven genera, six of which occur in South America, while the seventh (*Cercophonius*) is an inhabitant of South Australia and contains a single species. The families Scorpionidæ, Vejovidæ, Chærilidæ and Chactidæ have a distinctly "pentagonal" sternum with more or less parallel sides. The Chærilidæ belong exclusively to the old world. The Chactidæ are divided into three subfamilies, the European Euscorpiinæ and the neotropical Megacorminæ and Chactinæ, which have Mexico for their northern limit of distribution. The Vejovidæ are unevenly distributed between the Old World and the New. One of the eight genera composing this family is found on the shores of the Mediterranean (*Iurus,* with a single species *I. dufoureius*), another (*Scorpiops*) with about eight species in East India. Of the remaining six genera, two occur in South America, while the other four belong to the southern and western United States and to Mexico. The family Scorpionidæ, to which some of the largest scorpions belong, has representatives from various countries and regions. It is usually divided into five subfamilies. Of these the Urodacinæ are Australian; the Scorpioninæ Asiatic and African; the Hemiscorpioninæ Asiatic; the

Ischnurinæ African, Asiatic and neotropical (*Opisthacanthus elatus* is the single neotropical species); the Diplocentrinæ neotropical and Asiatic (a single genus with two species from eastern Asia).

All scorpions belonging to the large family Buthidæ have a distinctly "triangular" sternum with converging sides and truncated apex. The family is naturally divided into two large subfamilies. Of these the Buthinæ may be regarded as belonging to the Old World, since of its 14 genera a single genus and species (*Ananteris balzani*) is found in South America. The subfamily Centrurinæ includes four genera. The genus *Isometrus* is characteristic of the Old World but its commonest species, *I. maculatus,* is cosmopolitan and occurs in Florida, Hawaiian Islands, South America, etc. *Zabius* is South American. *Titius* is neotropical, although one species, *T. floridanus,* occurs in southern Florida. The largest genus, *Centrurus,* is represented by some of the commonest species in the southern United States and the subtropical and tropical America.

Let us fix our attention for a moment on the distribution and characters of two genera of scorpions common to the United States. One is *Vejovis* (of the family Vejovidæ) and is represented in this country by six species; the other is *Centrurus* (of the family Buthidæ) and is represented by seven species. *Vejovis* belongs more to the southwest and west. It is distributed through California, Nevada, Utah, Arizona, New Mexico and Texas and extending northward into Idaho and Nebraska. A single species, *V. carolinus,* is found in the southeast. It occurs as far north as South Carolina and spreads southward to the Gulf states and Texas. Except possibly this species, the other species of *Vejovis* occur also in Mexico where the genus is represented by four additional species which do not occur in the United States. I have besides, in my private collection, a new species of *Vejovis* from Terra del Fuego. *Centrurus* belongs more to the southeast. A single species (*C. exilicauda*) occurs in

California. *C. nigrescens,* a variety of the more southern *C. gracilis,* has been reported from Texas. Four species occur in Florida, but of these *C. gracilis* and *C. margaritatus* belong to a more southern fauna, the latter being the most common scorpion of Mexico and Central America. One species, *C. infamatus,* has practically the same distribution as *Vejovis carolinus,* spreading northward into South Carolina and southward into Texas and

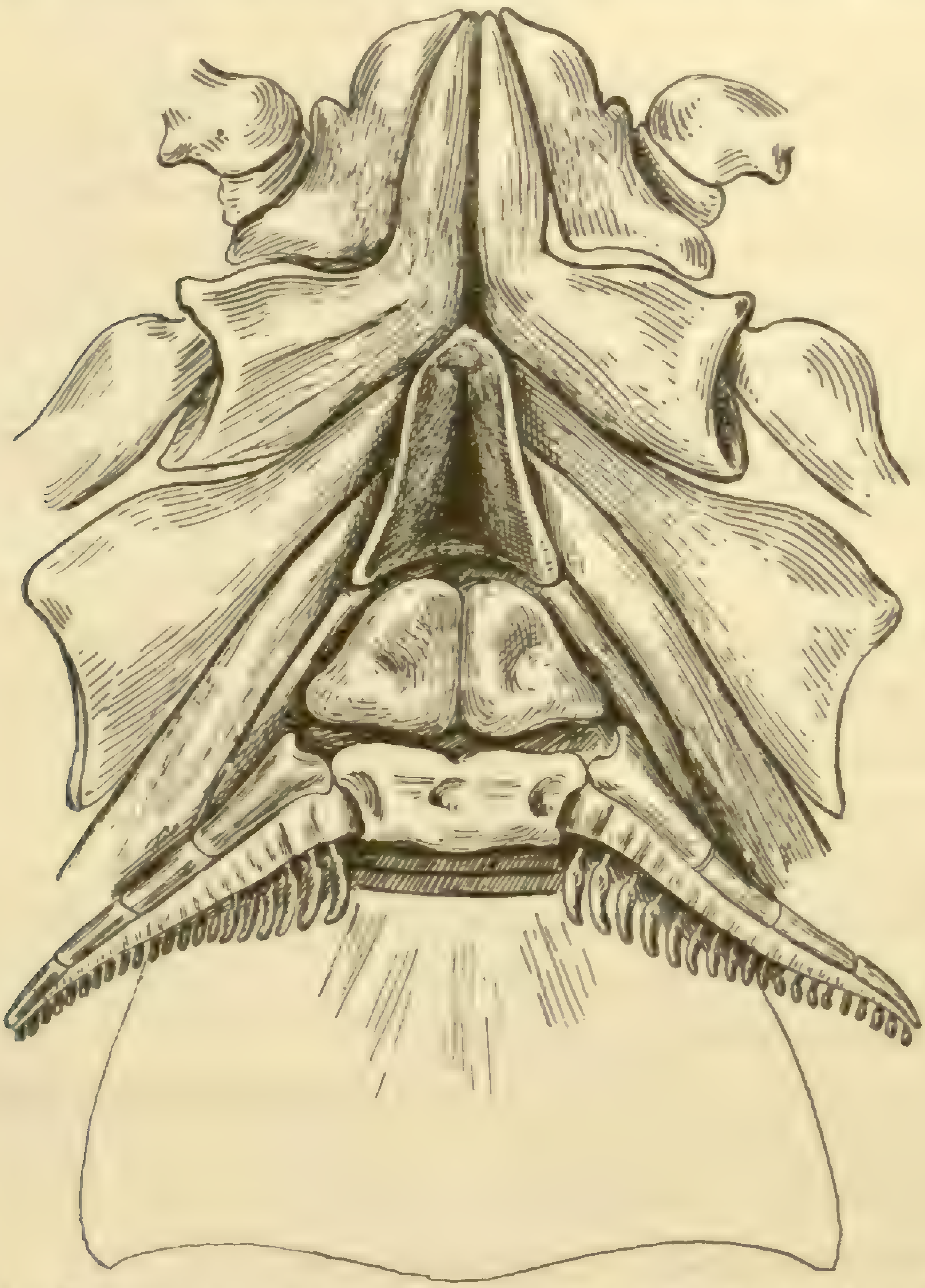

Fig. 1. Sternum, genital opercula, combs and coxæ of an adult *Centrurus insulanus* Thorell from Jamaica, W. I. The sternum is "triangular" with converging sides, but still shows traces of its original, pentagonal shape.

northern Mexico. *C. infamatus* is the common scorpion of the southeast and south. Several other species of *Centrurus* are characteristic of tropical North and South America.

Vejovis carolinus is a small scorpion, its length not exceeding 34 mm. The width of the carapace at the posterior edge is slightly less than the length. The center of the eye tubercle is about $\frac{2}{5}$ of the entire length of the carapace from its anterior edge. The sternum is pentagonal; the comb has 13–14 teeth; the central and inner rows of plates in the comb are beadlike. The fingers are rather short, being either as long as or only slightly longer than the hand.

Centrurus infamatus is usually about 45 mm. long, but large specimens measure up to 70 mm. The width of the carapace at the posterior edge is equal to or even slightly exceeds its length. The center of the eye tubercle is about $\frac{2}{5}$ of the entire length of the carapace from its anterior edge. The sternum is triangular. The comb usually has 18–19 teeth, although their number may reach 25. The central rows of plates is not beadlike, but composed of five plates the limits between which are difficult to ascertain. The inner row is beadlike. The fingers are rather long, being more than $1\frac{1}{2}$ times as long as the hand.

Let us now consider the Palæozoic scorpions. The sternum of the Silurian *Proscorpius osborni* Whitfield is unfortunately not preserved. Its nearest European relative, the Silurian scorpion *Palæophonus hunteri* Peach, has, according to Pocock, a pentagonal sternum. The sternum of the carboniferous scorpions is fairly well-preserved in many specimens. The family Isobuthidæ differs from all other fossil and recent scorpions in the position of the fourth pair of coxæ which abut against the genital opercula. The sternum is either triangular (*Palæobuthus*), rhomboidal (*Isobuthus*) or oval (*Eobuthus*). The family Cyclophthalmidæ has a "pear"-shaped sternum, the family Eoscorpionidæ a distinctly pentagonal one with parallel sides. If the pear-shaped and rhomboidal impressions of sterna do not owe their shape to poor preservation or displacements, then these types of sterna must have disappeared completely, as has the type of triangular sternum found in Isobuthidæ. Of

preserved fossils there remain then only the Silurian Palæophonidæ and the carboniferous Eoscorpionidæ having a sternum and arrangement of coxæ similar to that in recent scorpions. But the Silurian scorpions possessed other characters of their own and have either disappeared completely or perhaps have changed gradually into carboniferous forms. In the absence of Mesozoic fossils any attempt to trace relationships between recent and Palæozoic scorpions can be only conjectural. Thus in my "Monograph of Palæozoic Arachnida" I arrived at the conclusion (p. 26) that "the family Eoscorpionidæ shows many relations to the recent Scorpionidæ and Vejovidæ and represents probably two or three families thrown together for lack of distinctive characters." In formulating this opinion I was guided chiefly by the shape of the sternum, in several cases remarkably well preserved. Since that time I have made an observation, insignificant in itself, but one which affords an insight into the past history of recent scorpions possessing a triangular sternum and suggests a closer relationship between the Eoscorpionidæ and the Centrurinæ than between the former and the Vejovidæ. This observation was made by pure chance. While studying the problem of segmentation in Arthropods, I was examining a frontal section through a recently born *Centrurus insulanus* from Jamaica (Fig. 2). To my amazement the sternum proved to be beautifully pentagonal. An error of identification was excluded. I myself collected the material and preserved the adult females with the young carried on their back. Yet if objection should be raised, a final proof is offered by the fact that late embryos, too, have a pentagonal sternum and such embryos are easily obtainable from adult, gravid females. (Scorpions are without exception viviparous.) The young of *Centrurus infamatus* also have a pentagonal sternum, as have probably the young of all other species of the genus *Centrurus*. It is strange that no one has noticed this before, since there must be dozens of specimens in every museum. I find

an interesting confirmation of my observation in Fig. 10 of McClendon's paper on the nervous system of *Centrurus infamatus*.[1] The adults of his material were identified for him by no less an authority than Kraepelin, yet in the figure in question McClendon draws a distinctly pentagonal sternum in a surface view of a late embryo. The case is the more interesting because McClendon himself is unaware of the value of his observation, nor is

FIG. 2. Frontal section parallel to the ventral surface showing sternum, genital opercula, combs and coxæ in a very young ***Centrurus insulanus***. The sternum is distinctly pentagonal, with parallel sides.

there the slightest reference to it in the text. He simply drew the sternum as he saw it, without so much as mentioning it. An examination of the adults of *Centrurus insulanus* (Fig. 1) as well as of other species of *Cen-*

[1] *Biol. Bull.*, Vol. 8, 1904, p. 51.

trurus, shows that their sternum retains throughout life traces of its origin from a pentagonal prototype. Only the triangular apex is comparatively small and more or less hidden between the coxæ, and the sides of the sternum are strongly convergent, not parallel. The sternum in adults of various species of Buthinæ of the Old World shows also a small triangular apex hidden between the

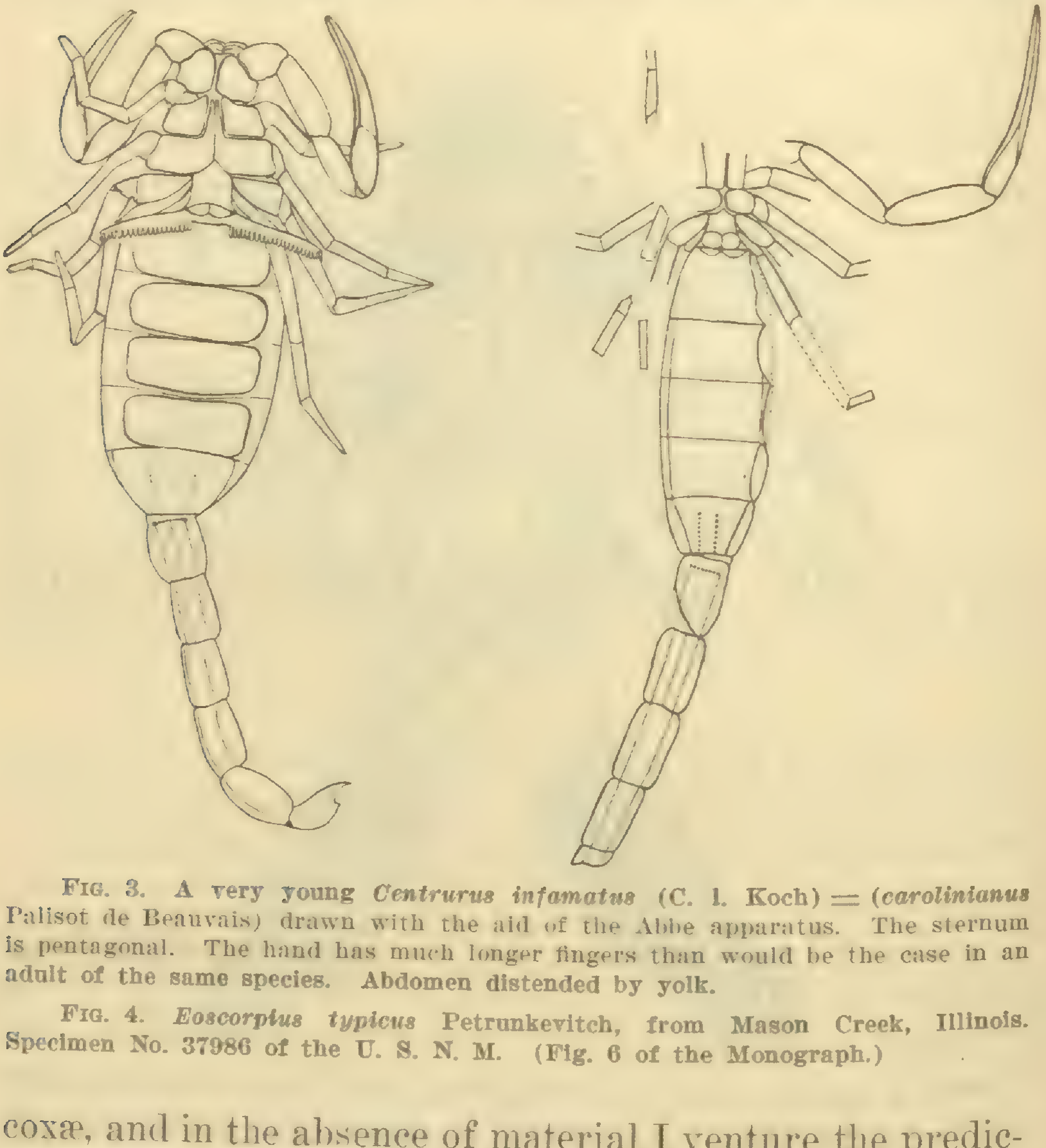

FIG. 3. A very young ***Centrurus infamatus*** (C. l. Koch) = (***carolinianus*** Palisot de Beauvais) drawn with the aid of the Abbe apparatus. The sternum is pentagonal. The hand has much longer fingers than would be the case in an adult of the same species. Abdomen distended by yolk.

FIG. 4. ***Eoscorpius typicus*** Petrunkevitch, from Mason Creek, Illinois. Specimen No. 37986 of the U. S. N. M. (Fig. 6 of the Monograph.)

coxæ, and in the absence of material I venture the prediction that the embryos will be proved to possess a pentagonal sternum.

Keeping this in view, let us now compare *Centrurus infamatus* with *Eoscorpius typicus* of the Pennsylvanic from Mason Creek, Illinois, a rather well-preserved Palæozoic scorpion of approximately the same size. Fig. 4

is taken from my monograph and represents the holotype of *Eoscorpius typicus* as it appears on the reverse. Fig. 3 is a careful drawing of a very young *Centrurus infamatus* made with the aid of an Abbe apparatus. This figure is much more enlarged than is Fig. 4, to facilitate comparison. The shape of the abdomen, as is well known, is the least constant character and we will leave it out of consideration. To avoid criticism, however, I would state that the great distension of the abdomen in the young *Centrurus* is due to the presence of embryonic yolk. When this disappears, the abdomen becomes much thinner. In older specimens the distension of the abdomen is frequently due to the growing embryos. Yet, in dissecting what I supposed to be gravid females, I was surprised to find no embryos in them and only small ovaries. The fact is that the distension of the abdomen is also often due to the condition of the liver.

The carapace has a fairly constant shape and is similar in both scorpions. For figure and measurements I refer to my monograph. I do not reproduce them here because the carapace of *Vejovis*, except for its size, is also of similar shape. The rows of granules on the caudal segments are not sufficiently well preserved in the fossil specimens to allow of a conclusion as to their exact number. On the other hand, the transverse row of granules at the anterior end of the first caudal segment, present in *Eoscorpius* and wanting in the adult *Centrurus*, is clearly defined in the young. The most interesting character is represented in the hand with its fingers. As a rule the ratio between the length of the fingers and that of the hand is a fairly constant one for each species. In the holotype of *Eoscorpius typicus* it is approximately 2:1; in the adult *Centrurus infamatus* it is 1.6:1, but in the late embryos and just born young it also approximates 2:1. With other words, *Centrurus* developed from an ancestor with relatively longer fingers and the trend of evolution was toward reduction in their length.

One character presents a difficulty. This is the comb.

Specimen No. 37987 of the U. S. Nat. Mus. of *Eoscorpius* shows a comb which is very broad at the base, and has, apparently, a single median plate, a beadlike inner row and 19 teeth. I identified this specimen as *Eoscorpius typicus* for the reason that "the general appearance of the specimen, the shape "of the tergites, especially of the seventh," strongly resembled the holotype. The specimen is incomplete and about twice as large as the holotype. Perhaps No. 37987 is after all of a different species. The shape of the comb in recent species of *Centrurus* is not always the same as in *infamatus.* The comb in *C. junceus* and *C. agamemnon* is twice as wide at the base as in the middle.

Taking all characters and the geographic distribution into account, we can not fail to notice the greater similarity between the young of *Centrurus infamatus* and *Eoscorpius typicus* than between the latter species and *Vejovis.* What advantage, if any, *Centrurus* has derived from the shortening of the fingers and the change in the shape of the sternum, is a totally different question which may possibly be answered by studying the functions and uses of these organs in recent species.

THEORIES OF HIBERNATION

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An examination of the literature on almost any particular natural phenomenon often reveals the fact that many different theories have been advanced to explain it. Some of these explanations may be mere opinions based upon no or but few scientific facts. One is also frequently struck with the immense literature that has been produced and the great gap that still intervenes between the accumulated facts and a clear understanding of the processes which they aim to elucidate, even after more than a hundred years of experimental work, which has usually been preceded by a much longer period of speculation by the great thinkers of the past. So that while we congratulate the last few generations upon the rapid growth that has been made in scientific knowledge, there yet remain phenomena that are almost as unintelligible to-day as they were a hundred years ago—the most earnest and often tedious experimentation and observations of several generations having shed but little light on the factors and mechanism involved.

The extremely interesting fact of hibernation (called "*Winterschlaf*" by the Germans, "*sommeil hivernal*" by the French and "*letargo*" by the Italians) illustrates well the above point. As is well known, during this dormant state the vital processes are greatly reduced. The changes that occur are especially marked in certain mammals, since they apparently undergo a rather sudden transformation from the warm-blooded (homoiothermal) type to the cold-blooded (poikilothermal) type. In the latter state such mammals are able to endure cold, deprivation of food, confined air, effects of many drugs, and other conditions that would be fatal at other times. Naturally

such profound physiological changes, in some respects almost as striking as the latent vitality of the seeds of plants and the spores of lower organisms, has aroused the attention of a great many observers. In fact, the literature on hibernation dates back to the time of Aristotle (384–322 B.C.), though real experimental work for the purpose of understanding the nature and cause of this torpid state, commenced with Conrad Gessner[1] (1551). From that date to the present there has accumulated a vast amount of data, the bibliography of which is now very accessible, due to the extensive works of Raphael Dubois,[2] published in 1896, and of Osvaldo Polimanti,[3] published in 1912.

As the exciting cause of so-called winter-sleep, cold has naturally received by far the greatest share of attention. A rapid survey of the subject shows that much difference of opinion has existed in regard to the manner in which cold acts and what other factors are involved. Buffon[4] (1749) and Lacépède[5] (1829) thought that the blood simply becomes cold when the small amount of heat produced by hibernating animals is not aided by the surrounding temperature. The cold blood then produces the changes characteristic of torpidity. Spallanzani[6] (1787), however, considered that he had experimentally demonstrated that the cold acts on the solid tissues of the body and not on the blood. According to him the lethargy is due either to the stiffening of the muscles or to the depletion of the cerebral blood vessels. On the other hand, Alibert[7] maintained that the cold diverts the blood from the periphery to the vessels of the brain and the resulting congestion causes torpor. But Serbelloni[8] (1866) claims to have found the vessels of the brain nearly empty in the case of three marmots in full hibernation. Hunter[9] (1775) and Serbelloni explained that the cold causes the animal to lose its appetite and in the absence of hunger, which is a stimulus, the animal retires.

A long list of authors, Daubenton[10] (1760), Geoffroy,[11] Cleghorn,[12] Allemand,[13] Carlisle[14] (1805), Barkow[15]

(1846) and others, have emphasized also the necessity of confined air or diminished respiration, Cleghorn and Allemand maintaining that this is the principal cause. Reeve[16] (1809) said that such a condition favors winter-sleep, while Bert[17] (1868) first concluded that lack of oxygen and then later[18] (1873) that the accumulation of CO_2 in the surrounding air might be the cause of dormancy. Mangili[19] (1807), however, denied that vitiated air has anything to do with this torpid state and Dubois[20] (1896) says that confined air is not necessary, for animals hibernate perfectly in well-ventilated places.

Marshall Hall[21] (1832) believed that the cold caused ordinary sleep, which diminishes respiration, and less heat is produced. Lessened respiration causes the blood to lose its arterial character and hence its power to stimulate the heart. The heart, however, changes its irritability so that it does not stop. This change in the irritability of the heart, then, is the important factor in hibernation. To him winter-sleep is something entirely different from the torpor produced by cold. To Edwards[22] (1824) and Legallois[23] (1824) sleep and cold are so bound up with heat production that a failure to keep up the body temperature causes torpidity to ensue.

Throughout the literature of the last one hundred years there is a strong tendency to consider hibernation as differing from ordinary daily sleep only in degree. Edwards[22] (1824), Dugé[24] (1838), Hall[21] (1832), Blandet[25] (1864), Patrizi[26] (1894), Dubois[27] (1896, 1910), Brunelli[28] (1902), Claparède[29] (1905), Allen Cleghorn[30] (1910) and Salmon[31] (1910) make definite statements regarding the striking similarity between ordinary daily sleep and hibernation. Gemelli[32] (1906) used the facts obtained by him from hibernating marmots, in disproving Salmon's theory of sleep. Indeed, it has been the hope of many of the students of hibernation to be able to throw some light on the process of diurnal sleep in man and other animals, by a study of what they have considered to be merely an extreme example of this physiological condition. The

discussions on sleep that appeared in the *British Medical Journal* in 1913 and the comprehensive treatise by Pieron[33] (1913) on the physiological problem of sleep, clearly indicate how little has been accomplished in this direction. Buffon[4] (1749), on the other hand, thought that ordinary sleep and hibernation were something entirely different. Monti[34] (1905) even now believes that these two forms of sleep have entirely different physiological meanings and that hibernation in its phylogenetic study should be compared with the dormancy of lower forms, as well as with ordinary sleep.

In reply to the question asked by the French Academy of Science over a hundred years ago as to the cause of this lethargy and why it pertains to certain animals, Saissy[35] (1808) stated that the cause fundamentally is to be found in certain anatomical peculiarities such as the enlarged character of the heart, central blood vessels, thorax, abdomen and cutaneous nerves, and the smallness of the peripheral vessels and lungs. To these he also added as important features the liquid quality of the blood and the sweetness of the bile. The diversion of the blood from the surface towards the center of the body, as a result of the external cold, dilates the heart and blood vessels of the thorax, and this interferes with respiration, thus decreasing heat production. As a consequence the animal becomes cold and numb. Prunelle[36] (1811), Barkow[15] (1846), Serbelloni[8] (1866) and Blandet[25] (1864) similarly believed in the importance of such—largely imaginary—morphological features.

Many investigators have associated hibernation with the nervous system. Claude Bernard[37] (1855–76) thought that the cold acts on an unusually well developed peripheral nervous system, and by slowing respiration cools the body. This is a loss of stimulus to the heart and muscles and torpor results. Reeve[16] (1809) stated that cold acts on a special organization of the nervous system, which causes diminished respiration, etc.; while Quincke[38] (1882) interprets the facts he and others have observed,

in connection with the marmot, as indicating the existence of a heat center in the brain through whose influence on the various organs of the body, metabolism and heat regulation are so affected as to produce winter-sleep. The altered respiration and circulation are secondary results. Dutto[39] (1896) is also inclined to believe that hibernation strictly depends upon the regulative influence of the nervous system upon metabolism and thermogenesis. He further considers that the marmot has the power to emit more heat than has the rabbit, so that torpor may be based upon the difference in the power of the integuments of hibernating and non-hibernating animals to lose heat. Merzbacher[40] (1904), after reviewing much of the literature dealing particularly with the rôle of the external temperature, food and the nervous system in the production of winter-sleep, concludes that the external cold is only a secondary aid. Cold, like abstinence from food, immobility, slower respiration and lack of oxygen, simply makes it easier for the animal to cool off and remain cold, and tends to make the sleep more profound. The essential characteristic of the hibernating animal, as compared with the non-hibernating animal, according to him, is its ability to change at a rather definite period and in a comparatively short time from the homoiothermal to the poikilothermal type and again at the end of hibernation to return rather abruptly to the former condition. The explanation of both of these alterations, he thinks, is probably to be found in a certain nervous mechanism in the mid-brain and medulla which is capable of influencing respiration, circulation and metabolism, and, in short, the production and loss of heat. The other changes characteristic of the lethargy are natural consequences of and adaptations to the hypothermic and hypofunctional condition.

In addition to other internal factors there is, according to Barkow[15] (1846), a special susceptibility to the external cold due to a rather primitive organization of hibernating animals. Noè[41] (1903) thinks that a primitive

structure of the organism is the important cause of the lethargy; but it acts as a mechanism of economy by increasing the resistance of the animal to cold, rather than to starvation, and thus prevents histolysis from reaching a dangerous point. An inefficient heat-regulating mechanism has been considered the true explanation of winter-sleep by such men as Dugès[24] (1838), Marès[42] (1889), and Polimanti[43] (1904). Simpson[44] (1911) in this laboratory has shown that the woodchuck can not be said to ever have a normal temperature in the sense that a homoiothermal animal has. Merzbacher[40] (1904) cites many cases similarly indicating the weak thermogenic organization among winter-sleepers. Recently Polimanti[45] (1914) has explained his views concerning this labile thermogenic organization. To him it is due to the fact that at some remote period all animals then existing periodically fell into lethargy. With evolutionary development most mammals and all birds lost this ability. Hibernating animals, however, are still able to return to this cold-blooded type when the heat-producing reflexes fail, which they are apt to do when the cold becomes extreme. Marès[46] (1913) holds fundamentally this same view—a view he advanced in 1889. He says that the cause of hibernation is in the organism itself. He regards the facts presented by Pembrey[47] and Babák[48] and others concerning the poor heat-regulating mechanism of the newborn in man and other animals, as well as those by Merzbacher[49] on the return of the nervous system to a more segmental type during winter-sleep, as strong evidence in favor of the theory, and as indicating that hibernating animals merely revert to a more primitive type in which there is no specific sensibility to the outer cold, *i. e.*, in which no specific heat-regulating reflexes are called forth by the external temperature. He further thinks that since the weakness is in the nervous system, it ought to be possible to bring about some of the conditions of torpor by means of hypnotic suggestions. He and Hellich[50] (1889) succeeded by this means in getting a fall of

2.5° C. in the body temperature of a hysterical woman. Others have gone much farther in this regard. Thus Liébeault[51] (1866) and Forel[59] (1877), especially the latter, consider hibernation similar in nature to hypnotic sleep. To Marès, however, the initial cause of winter-sleep is the ability of the nervous system to loose its specific sensitiveness to the external cold. This sensitiveness, he thinks, does not belong to the fundamental properties of the nervous system, since it is not found in the young undeveloped animal. It is a property acquired slowly ontogenetically just as it was slowly acquired phylogenetically by the two highest classes of animals. A similarity between the hibernating and fetal states was noted long ago by Pallas[53] (1778), Prunelle[36] (1811), Tiedermann[54] (1815) and Edwards[22] (1824). Tiedermann claimed that in both states there is merely a vegetative existence, hardly any appreciable difference between the appearance of the venous and arterial blood, much serum and little clot when the blood coagulates, a low body temperature, an enlarged thymus (he included the hibernating gland as part of the thymus) containing a fluid, and a secretion of bile. He therefore considered winter-sleep as a periodic return to a fetal state. Pembrey and Hale White[55] (1896) regard the evolution of hibernation, not as the acquisition of a new power, but as a retention of one already present, as is evident from the condition of young mammals and birds in whom the heat-regulating power is inefficient.

Many observers have questioned the value of cold as a factor in the production of this dormant state. Quincke[38] (1882) thought that rest and an appropriate temperature generally, though not always, cause torpor, and yet he said that there seems to be some relationship between degrees of lethargy and external temperature. Blandet[25] (1864) considered that winter was only occasionally, if at all, the cause; while Horvath[56] (1872–81), with whom Bunge[57] (1901) seems to agree, said that hibernation is not sleep at all and that winter has nothing to do with it.

Hahn[58] (1914) concludes that the torpid condition is not dependent upon cold weather, although his thirteen-lined ground squirrel usually hibernated with each cold spell and woke up with the return of warm weather. Experimentally it was early shown that cold will not induce typical lethargy. Thus Buffon[4] (1749) in the case of the hedgehog, Daubenton[59] in the hamster, Hunter[9] (1775) in the dormouse, Mangili[19] (1807) and Bossi[60] in the marmot, Horvath[56] in the spermophile and hedgehog and Marès[42] (1892) in the spermophile, have failed to induce true hibernation by exposure of the animal to low temperatures. Saissy[35] (1808) is supposed to have produced winter-sleep by continued cold and confined air; but like some other reported cases of artificially produced torpor, it is not clear that the experimentally produced state was the same as true hibernation. Sacc[61] (1858) after eight years of observation on the marmot could see no relationship between the condition of the atmosphere and winter-sleep. Mills[62] (1892) found that while bats could be worked like a machine by varying the temperature, marmots, on the contrary, showed a surprising indifference to the surrounding temperature. Berthold[63] (1837) claims that dormice became dormant in a room kept warm (16° C.) all winter, though torpidity was delayed two months. Merzbacher[46] (1904) mentions similar experiences of his own with a bat, as well as several other comparable cases. Mangili[19] (1818) saw a dormouse fall into lethargy in the month of June and not wake up till the middle of July. Forel[52] (1887) records that two dormice which remained awake and active all winter, became torpid in May and remained in this condition till August in spite of the great heat. Marès[42] (1892) found that some spermophiles and hamsters may hibernate in September at 16° C. while others remain awake all winter although the thermometer falls below zero. Hence he concluded that cold does not cause winter-sleep. Valentine[64] (1857), Horvath[56] (1881) and Quincke[38] (1882) have observed marmots become dormant during the summer. Hence

Pembrey[65] (1898), while recognizing that want of food and cold seem to be the most important factors in hibernation, says that some other condition yet unknown is necessary to explain such lethargy during the summer.

As a result of the uncertain action of cold, certain other external conditions have been considered the real exciting cause of hibernation. The food factor was emphasized by Mangili[19] (1807), who believed that neither cold nor vitiated air has anything to do with the production of this torpid state. He thought fasting was necessary because, of several animals under the same external conditions, those animals that were fed did not become dormant, while the non-fed ones did. Marshall Hall[21] (1832) stated that the lack of food predisposes the animal to torpor by rendering it more susceptible to cold. Sacc[61] (1858) concluded that, while he could see no relationship between the atmosphere and torpidity, he could see some connection between the fatness of the animal and the length and profoundness of winter-sleep. He, therefore, concluded that obesity, in connection with fatigue, is the cause of hibernation. Claparède[29] (1905) and Forel[52] (1887) think that the amount of fat may be an important factor, while Beretta[66] (1902) opposes this idea. Simpson[67] (1912) finds that feeding woodchucks greatly interferes with winter-sleep, at least in captivity. Albini[68] (1901) in case of the marmot, and Reeve[16] (1809) in connection with dormice and hedgehogs, also confirm the observation of Mangili on the rôle of food in preventing hibernation. Yet it appears that these animals (marmots) may go into winter sleep while plenty of food is available. Thus Mills[62] (1892) found that during the winter of 1890–91 a marmot hibernated in a cage provided at all times with plenty of food; but during the two following winters two other marmots, kept in the same room and in the same cage under similar conditions, did not hibernate at all, though the temperature got low enough to freeze the water in the cage. It is also a common observation that some of these animals naturally retire

while food is plentiful. Allen Cleghorn[30] (1910) questions the lack of food as a factor in producing lethargy because spermophiles and marmots hide away for winter when their food supply is at its best. In British Columbia he finds that these animals retire a month earlier in the lowland than at the timber line because, he thinks, in the latter region they have not had time to acquire enough fat, since at the timber line they come out of hibernation later in the spring. Thus it is not clear exactly what part food plays in the production of this dormant state.

Treviranus[69] (1802) said that the cause of torpidity during winter lies in the ability to live with all the vital processes at a minimum. This is an acquired character resulting from the habit of sleeping during winter, as is evident, he thought, from the fact that it is lost in marmots kept in captivity. The earlier opinion of Barton[70] (1799) was that it is an accidental circumstance and not a specific character. The general idea, however, that some sort of instinct, in connection with other factors, is involved, was held by Reeve[16] (1803), Barkow[15] (1846), Claparède[29] (1905) and others. Desjardine[71] (1843) thought that the need for sleep in rodents is as great as the necessity of migration in birds. Blandet[25] (1864) described winter-sleep as a relic—an echo from remote periods when this phenomenon was general, having developed as a result of winters so severe that unless this conserving process was resorted to, the animals would have perished. Hibernation is thus, according to this author, the effect of habit and annual periodicity. It still persists in certain animals, but will soon become extinct. Brunelli[28] (1902) believes that this tendency is the result of a long period of evolution favored by the nature of the burrow, etc., where hibernation takes place. But according to Albini[68] (1894) the factors aiding this evolution are not remoteness or other conditions of the burrow, but the immobility of the animal. Carlier[72] more recently (1911) classifies hibernation with estivation (summer-sleep) and migration. Winter-sleep in mammals like the instinct

of migration in birds, he thinks, may have developed in remote ages, the prime cause being want of food, and not cold.

Dubois[73] (1895) has developed a *carbonic auto-narcosis* theory according to which hibernation is due to the accumulation of CO_2 in the blood and tissues of the animal. This excess of CO_2 is supposed to cause a form of narcosis as seen in the torpid condition of the hibernating animal. When the CO_2 reaches a certain concentration the respiratory center is excited, respiration accelerated, and the muscles become hyper-irritable. These culminating results are responsible for the awakening from dormancy. The author claims that he can induce typical hibernating sleep by causing the active marmot to breathe a mixture of air (42 per cent), CO_2 (45 per cent) and oxygen (12 per cent). Torpid marmots remain dormant if supplied with this mixture. By increasing the proportion of CO_2 respiration is accelerated, and if the supplying of CO_2 is continued the hibernating marmot wakes up. The CO_2 is supposed to act principally on a nervous center for sleep situated in the mid-brain, since marmots deprived of cerebral hemispheres are able to sleep and wake up; but with only the medulla intact they are unable to awake. Further, Dubois[74] (1894) found that CO_2 actually accumulates in the blood during hibernation in the marmot and decreases again when the animal wakes up. Such an increase in the CO_2 content of the blood during hibernation has just been observed in this laboratory in case of the woodchuck (*Marmota monax*).[75]

Upon sufficiently good authority[76] to receive the serious consideration of such an author as Max Verworn,[77] certain ascetics of India, known as fakirs, are able to voluntarily go into a condition of almost suspended animation not unlike hibernation in some respects. While in this condition it appears that these fakirs may be buried three or four feet in the ground for days, or may be inclosed for six weeks without food and with but little air in a tight box which in turn is sealed up in some dark inner room.

When disinterred the body is cold and stiff with no signs of any pulse, and apparently lifeless; but it revives with no bad after-effects upon the application of warm water to the head and after being manipulated for a quarter of an hour. Dubois emphasizes the fact that in order to induce this state of trance, the fakirs make it a point to breathe as little as possible. This and much other indirect evidence is brought forward by this author in support of his carbonic auto-narcosis theory of hibernation.

Mosso[78] (1899) holds just the opposite view. He thinks that winter-sleep is due to a condition of acapnia, or lack of CO_2 in the system.

It is not strange that in this age of ductless glands and internal secretions some theory should be brought forward that would involve the ductless glands. In 1905 Salmon[79] advanced the view that the pituitary body (hypophysis cerebri) is a center for sleep and produces an internal secretion which by virtue of some vasomotor or autotoxic power acts on the nervous system and thus produces normal sleep. His view has been further elaborated in later publications[80] (1910) in which he states that hibernation may be explained upon an analogous mechanism involving especially the so-called hibernating gland —a structure which has lately received renewed attention by physiologists. Salmon seems to favor the old idea that a depletion of the cerebral blood vessels offers the best explanation of the lethargy characteristic of the hibernating state. The rôle of the hibernating gland, however, is very uncertain. This structure is now generally regarded as reserved food. Vignes[81] (1913), however, considers it probable that it plays some important physiological rôle, particularly in hibernation, since its extirpation in the white rat, where the operation is attended with little difficulty, is nearly always fatal. He finds that this structure modifies the action of certain toxic substances such as adrenalin, chloroform, tetanus toxin and cobra venom, retarding the action of some and accelerating that of others. He further maintains that

this gland contains lipase, and while it does not convert starch to sugar, its extirpation diminishes the amyloptic power of the serum. It also has an antitryptic power. Thus he conceives that it might serve as an economizer of proteins by insuring the utilization of reserve carbohydrate and fats during the long period of winter-sleep.

Salmon's view on the rôle of the hypophysis cerebri in the production of sleep was soon criticized by Gemelli[32] (1906), who argued that if this hypothesis were correct, the pituitary body would show signs of increased activity during hibernation, since, as has already been stated, hibernation is considered by many to differ from ordinary diurnal sleep only in degree and duration. But on the contrary, he found that the cyanophil cells of this gland in the marmot decreased during winter-sleep and that they increased again simultaneously with the appearance of numerous karyokinetic figures after the animal wakes up in the spring. Gemelli interpreted his findings as indicating that the anterior lobe of this organ cooperates with other ductless glands in neutralizing toxins which are produced in increased quantity when the animal becomes active, and hence is not to be regarded as a center of sleep. A later contribution to the relationship between the pituitary body and hibernation is by Cushing and Goetsch[82] (1913). As a result of observations on the hypophysis of the woodchuck, in which they confirm in general the findings of Gemelli on the decrease in size and histological changes during winter-sleep, these authors suggest that hibernation may be ascribed to a period of physiological inactivity, possibly of the entire ductless gland series, but certainly more especially of the pituitary gland, because during the dormant period this structure diminishes in size and shows profound histological changes and because deprivation of this gland in the human subject and in experimental animals causes a train of symptoms comparable to those of hibernation. Mann[83] (1916), however, found demonstrable changes in the pituitary body and other ductless glands of a large num-

ber of ground-squirrels (*Citellus tridecemlineatus*) to be absent or so inconstant, especially at the critical period—at the onset of hibernation—that the assumption of any theory ascribing the phenomenon of hibernation to a lack of function of all or any one of the ductless glands is not justified.

From this general summary it will be seen that great diversity of opinion prevails regarding the immediate cause of this extremely interesting condition, and of the sudden transformation from the homoiothermal to the poikilothermal state (and vice versa) so characteristic of hibernating mammals. It is not the author's object, however, to discuss the relative merits of the various theories. Suffice it to say that all of them are based upon insufficient data. To say which of the various conditions associated or occurring simultaneously with winter-sleep are concerned with the production of the lethargy and which are the results of this or some other condition, is extremely difficult. Until certain causal relations are definitely established between the factors concerned, many of these theories are of very little value except as a stimulus to further research. It is thus very evident that we are far from having any adequate explanation of the mechanism of this phenomenon, to say nothing of how it was established as a more or less variable character in certain animals.

If hibernation of mammals is only an extreme form of ordinary diurnal sleep of man and other animals, it is especially to be hoped that this subject will continue to be investigated by more modern and adequate means, for no entirely satisfactory theory has yet been advanced to explain the physiological cause of ordinary sleep. Since winter-sleep may also be attended with total abstinence from food and drink for many months, the facts derived from a study of the various conditions associated with this dormant period are of interest also in connection with the subject of inanition in particular and metabolism in general, as is plainly indicated by the frequent reference

to and comparison with the observations on hibernating animals found in the literature on inanition.

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SHORTER ARTICLES AND DISCUSSION

VARIATION, CORRELATION AND INHERITANCE OF FERTILITY IN THE MAMMALS

THE purpose of this review is to give an outline of the problems of fertility in the mammals (exclusive of man) which may be solved by the application of biometric formulæ to statistical data, to furnish an index to the available statistics, and to indicate the results to which the statistical analyses of the raw observations have led. Many of the biometric constants are published here for the first time.

TYPE AND VARIATION IN FERTILITY

The most fundamental biological questions which can be asked concerning series of data on fertility *considered quite independently of any other characteristic of the organism, its ancestry or its environment* are three:

(*a*) What is the typical and average fertility of different species or races?

(*b*) What is the variation, within the race, in reproductive activity as compared with that of variation in the degree of development of somatic characters?

(*c*) May fertility, like the bodily attributes of organisms, be described by mathematical curves?

With more comprehensive data concerning other characteristics of the individual organism, its ancestry or environment, more varied problems may be investigated, but none of more fundamental importance.

With respect to (*a*) it need only be said initially that biologically a knowledge of the number of offspring characteristic of a species has the same importance as a knowledge of any other of its peculiarities. That species may differ widely in fertility as in other characteristics is obvious without the collection of extensive statistics or the application of mathematical formulæ. It is only in a consideration of relatively *slight* differences in fertility in *nearly related* species or races or in individuals of the same race existing under various conditions, that biometric

work became indispensable. It is just here, too, that the purely descriptive significance of fertility gives way to genetic, economic and sociological sources of interest.

As yet information on these subjects is all too meager. Lloyd[1] has emphasized slight differences in fertility in species formation in the rodents. Donaldson[2] has brought together the available data for fertility in the rat. For swine, Rommel[3] and Bitting[4] have given extensive data for different breeds and periods. Further records are available for swine from the studies of Wentworth and Aubel to be discussed below. Equations for theoretical curves of distribution of number of young per litter in Rommel's series have been worked out by Surface.[5] Large masses of statistics have been extracted from the herd books for sheep by Rietz and Roberts. Taken altogether, only a beginning has been made in a field that has not merely great biological interest, but in certain of its bearings is of material economic importance.

The most extensive and exact work on differences in fertility has been done on man, but a discussion of this subject falls outside the scope of the present review.

Since data for the solution of the problems of group (*a*) are as yet inadequate, it is idle to attempt any detailed discussion of those of group (*b*) and (*c*). Data for such purposes are, however, now becoming available much more rapidly than heretofore.

Birth Order and Litter Size

Fairly large series of records showing the relationship between birth order and litter size are now accessible.

Minot[6] has given data for the relationship in guinea pigs. The averages which may be computed from these are:

Order of Litter	F	Mean Size
First	51	1.96
Second	29	2.97
Third	15	2.80
Fourth	4	3.50

[1] Lloyd, R. E., "The Inheritance of Fertility," *Biometrika*, 8: 244–247, 1911.

[2] Donaldson, H. H., "The Rat," pp. 22–23, 1915.

[3] Rommel, G. M., "The Fecundity of Poland China and Durac Jersey Sows," Circ. U. S. Dep. Agr., Bu. Anim. Ind., 95, 1906.

[4] Bitting, A. W., "The Fecundity of Swine," Ann. Rep. Ind. Agr. Exp. Sta., 10: 42–46, 1897.

[5] Surface, F. M., "Fecundity of Swine," *Biometrika*, 6: 433–436, 1906.

[6] Minot, C. S., "Senescence and Rejuvenation," *Jour. Phys.*, 12: 97–153, 1891.

Crampe[7] many years ago showed from his extensive data on rats that the maximum fertility was on the second litter. King and Stotsenberg[8] have recently given data which lead to the following averages:

Order of Litter	F	Mean Size
First	21	6.24
Second	21	7.71
Third	18	7.06
Fourth	15	6.40

Pearson and Weldon have shown[9] that in mice there is an increase in the mean number of young from the first to the third litter, thus:

Order of Litter	Mean Offspring
First	5.46
Second	5.57
Third	5.76

For the rabbit Bailey, *fide* Hammond,[10] gives the values:

Order of Litter	Mean Offspring
First	5.58 ± 0.32
Second	7.25 ± 0.41
Third	7.08 ± 0.38

Such data as these are of obvious importance in the physiology of reproduction in the mammals. They will be of far greater value when it is possible to determine the influence of the actual age of the mother as well as of the order of birth upon fertility. Detailed records of size as well as of number of offspring and of mortality would also be of great importance.

Relationship between Fertility and Somatic Characters

The interrelationship between fertility and somatic characters is a subject which may have a morphogenetic, genetic or economic interest.

Reference to some of the earlier literature has already been

[7] Crampe, H., "Zucht-Versuche mit zahmen Wanderratten. I. Resultate der Zucht in Verwandschaft," *Landwirths. Jahrb.*, 12: 389–449, 1883.

[8] King, H. D. and J. M. Stotsenberg, "On the Normal Sex Ratio and the Size of the Litters in the Albino Rat (*Mus norvegicus albinus*)," *Anat. Record*, 9: 403–420, 1915.

[9] *Biometrika*, 7: 384, 1910.

[10] Hammond, J., "On Some Factors Influencing Fertility in Domestic Animals," *Jour. Agr. Sci.*, 6: 263–277, 1914.

made[11] in a memoir dealing with plant materials and certain special problems more minutely analyzed on further sets of data.[12]

In the mammal, the relationship between fertility and somatic characters may be determined from (*a*) the somatic characters of an individual mother and her fertility, or (*b*) the characteristics of the progeny which serves as the measure of the fertility of a mother. Obviously, these two methods of operation are biologically not at all comparable.

The economic importance of the possible correlation between bodily characteristics and fertility has naturally given rise to many popular beliefs concerning the existence of such a relationship. Wentworth and Aubel[13] have, however, found no evidence of such in a comparison of the mean litter size in "large type" and "small type" hogs.

Pearson has shown[14] from Captain Lloyd's data[15] that there is a sensible and almost linear relationship between weight of mother and number of young in litter in Poona and Belgaum rats. The intensity of the correlation is, however, low, of the order $r = .160$.

Data for the full interpretation of such relationships are much needed but not as yet available. The problem is evidently one of great complexity. As Pearson points out, at certain stages of pregnancy the number of young might actually influence, by its own contribution, maternal body weight.[16] Furthermore, in these rodents growth continues notwithstanding pregnancy, and one might expect some correlation between weight of mother and size of litter as a resultant of the relationship between the age of the mother and her own weight and the age of the mother and the size of her litter.

Minot found that the over-gain in weight of pregnant guinea pigs is not all lost after delivery[17] and Watson[18] many years ago

[11] Harris, J. Arthur, *Biometrika*, 8: 52–65, 1910.

[12] Harris, J. Arthur, *Amer. Jour. Bot.*, 1: 398–411, 1914.

[13] Wentworth, E. N. and C. E. Aubel, *Jour. Agr. Res.*, 5: 1148, 1916.

[14] Pearson, K., "Darwinism, Biometry and Some Recent Biology," I, *Biometrika*, 7: 368–385, 1910.

[15] Lloyd, R. E., "The Relation between Fertility and Normality in Rats," *Rec. Ind. Mus.*, 3: 261–265.

[16] Minot (*Journ. Phys.*, 12: 141–145, 1891) has shown that in the guinea pig there is a relatively enormous over-gain in weight before delivery.

[17] Crampe (*loc. cit.*) has given certain data on the weight of mothers after the first and second deliveries in the rat.

[18] Watson, J. B., "The Effect of the Bearing of Young upon the Body

adduced evidences to show that females which have borne young are heavier than unmated controls. Whether the effect of bearing young is cumulative in such a way as to influence the correlations in Captain Lloyd's series is not yet evident.

Taking these various factors into account, there seems little ground for believing that there is any material correlation between the fertility of a mammalian female and her measurable somatic characters.

There is an obvious physiological and morphogenetic interest attaching to the correlations between the number of individuals born in a litter and the characteristics of these individuals.

Consider first the correlations between number of pigs in the litter and number of nipples, in swine. For Parker's[19] and Bullard's data the values are:

For males, $r = .0810 \pm .0121$,
For females, $r = .0324 \pm .0124$.[20]

These are numerically low, but both are positive, and may possibly be significant in comparison with their probable errors. They may indicate morphogenetic relationships between the vigor of the mother as indicated by the number of her young and the characteristics of these young.

These positive correlations for number per litter and number of nipples are of interest in connection with the *negative* correlation for number in the litter and mean weight of individuals suggested many years ago by Minot,[21] who states that in guinea pigs the size of the animals at birth depends to a considerable degree upon the number of young in a litter: the larger the litter the smaller the animals at birth. Fortunately Minot has given data from which approximate[22] values of the correlation between number of individuals per litter and birth weight may be compared. The results are:

Weight and the Weight of the Central Nervous System of the Female White Rat,'' *Jour. Comp. Neur. Psychol.*, 15: 514–524, 1905.

[19] Parker, G. H. and C. Bullard, ''On the Size of Litters and the Number of Nipples in Swine,'' *Proc. Amer. Acad. Arts and Sci.*, 49: 399–426, 1913.

[20] Parker and Bullard give the correlation $r = .0035 \pm .0124$ for females only. This is evidently erroneous. Both values given here have been calculated from their data.

[21] Minot, C. S., ''Senescence and Rejuvenation. I. On the Weight of Guinea Pigs,'' *Jour. Phys.*, 12: 96–153, 1891.

[22] The only difficulty lies in the fact that his Tables VII and VIII do not contain the same number of individuals.

$$\text{For males, } r_{nw} = -.437 \pm .039,$$
$$\text{For females, } r_{nw} = -.431 \pm .044,$$
$$\text{For all, } r_{nw} = -.430 \pm .029.$$

The results for males and females are in remarkable agreement. Evidently there is a large influence of the number born upon the weight of the individual.[23]

If the results be expressed in terms of regression of weight of individual upon number in litter the equations are:

$$\text{For males, } w = 87.626 - 5.214\,n,$$
$$\text{For females, } w = 84.375 - 4.741\,n,$$
$$\text{For all, } w = 86.006 - 4.960\,n.$$

King[24] has given direct evidence for the influence of weight of mother in the weight of the young at birth.

> Very young females and those that have passed their prime have smaller litters, as a rule, than females at the height of their reproductive powers.

And again,

> The body weight of a female influences the birth weight of her young chiefly because it depends on the two more important factors of age and physical condition.

Finally it may be noted that in the case of sheep the size at birth and rate of development of twin and triplet as compared with the single lambs is a problem of very real economic importance. Both Bell and Marshall have considered this phase of the question. Unfortunately no extensive quantitative data are available for analysis on this point.

Inheritance of Fertility

Biologically all recent studies on the inheritance of fertility differ from the classic memoir of Pearson, Lee and Bramley-

[23] That factors other than number per litter may profoundly influence birth weight may be seen at once by determining the correlation between the weight of the individual pigs born in litters of two as given in Minot's Table XI. Using symmetrical tables I find for the correlation between the weight of the two individuals

$$r_{w_1 w_2} = .686 \pm .046.$$

This similarity in weight is probably due in part to hereditary and in part to environmental factors.

[24] King, H. D., "On the Weight of the Albino Rat at Birth and the Factors that Influence It," *Anat. Rec.*, 9: 213–231, 1915.

Moore[25] on fertility in man and fecundity in race horses in that they deal with the number of young produced at a single birth instead of with the total young produced during the reproductive period or the ratio of the number of young actually born to the number which might have been produced under the circumstances.

For Poland China sows Rommel[26] and Rommel and Phillips[27] found values of the correlation between the size of litters in which dam was farrowed and size of litters produced by daughters ranging from .1088 to .0032, the values decreasing with moderate regularity as the daughters became older. For all ages they find the correlation $r = .0601$, and conclude that fertility is slightly but definitely inherited.

George (*fide* Wentworth and Aubel, *loc. cit.*) worked out four supplementary series in Poland China swine with the results:

Daughter and dam,	$r = .0615 \pm .0390$,
Dam and grandam,	$r = .1147 \pm .0343$,
Daughter and maternal grandam,	$r = .0025 \pm .0392$,
Daughter and paternal grandam,	$r = .0508 \pm .0392$.

All of these values are positive, but they are very small and no one of them may be considered statistically trustworthy in comparison with its probable error.

Weldon and Pearson[28] give a series of six relationships, both parental and grandparental, for size of litter in mice, with the result that no correlation whatever could be demonstrated.

Wentworth and Aubel[29] have considered the possibility of the segregation of litter size in the two first descendant generations of matings between boars and sows farrowed in litters of various sizes by determining the standard deviation of the number per litter in the so-called F_1 and F_2 generations. Let l be the num-

[25] Pearson, K., A. Lee and L. Branley-Moore, *Phil. Trans. Roy. Soc. Lond.*, *A*, 192: 257–330, 1899.

[26] Rommel, G. M., "Inheritance in the Female Line of Size of Litters in Poland China Sows," *Biometrika*, 5: 203–205, 1906.

[27] Rommel, G. M., and E. F. Phillips, "Inheritance in the Female Line of Size of Litter in Poland China Sows," *Proc. Amer. Phil. Soc.*, 45: 245–264, 1906.

[28] Pearson, K., "On Heredity in Mice from the Records of the Late W. F. R. Weldon. I. On the Inheritance of the Sex Ratio and of the Size of Litter," *Biometrika*, 5: 436–449, 1907.

[29] Wentworth, E. N., and C. R. Aubel, "Inheritance of Fertility in Swine," *Jour. Agr. Res.*, 5: 1145–1160, 1916.

ber of pigs in the litter in which an individual was farrowed, d the number of pigs in the litter in which its dam was farrowed, and S and D the numbers in the litters in which the grandsire and grandam were farrowed. Then, the authors reason, if fertility be due to factors which differ in the grandsire, S, and the grandam, D, and if Mendelian segregation occurs in the fashion assumed by several of these who have worked on quantitative characters, one should expect the mean value of the standard deviation of litter size in cases in which D and S differ widely to be higher than the mean value in cases in which they are closely similar. There is no conclusive evidence of such greater segregation in the F_2 from dissimilar grandparents.

Now the data published by Wentworth and Aubel permit the consideration of several additional questions of considerable interest in connection with the problem of the inheritance of fertility. Thus from the mean litter sizes in their Table II and the distributions of litter size in the three generations in their Table IV, it is quite possible to calculate *approximately*[30] correct correlations for the relationship between size of litter in different generations. Thus the formula:

$$r_{xy} = \frac{\Sigma(xn\bar{y}_x) - [\Sigma(x)/N][\Sigma(n\bar{y}_x)/N]}{\sigma_x \sigma_y},$$

where the bars denote the means of the y (descendant) litters associated with particular, x, classes of ascendant litters, leads to the values:[31]

$$r_S = .071 \pm .023, \quad r_{Dd} = .126 \pm .022,$$

$$r_{Sl} = .120 \pm .022, \quad r_{Dl} = .100 \pm .022.$$

Superficially considered, these values seem in excellent agreement with those published by Rommel and others, but the fact that r_{Sd} has a value which is possibly significant statistically, should at once arouse suspicion, for surely there is no genetic reason (excepting possibly non-viability of sperm or the production of duplicate twins through an influence of the sperm upon the egg) why there should be a correlation between the size of the litter in which a boar was farrowed and the size of litter in which his daughter was farrowed. Mistrust is heightened by the fact

30 Unfortunately there are inconsistencies in these tables which show the existence of typographical errors precluding exact constants.

31 Unfortunately data for the determination of r_{di} are wanting.

that r_{Sd} is actually though perhaps not significantly lower than r_{Sl}, whereas on the female side $r_{Dd}r > r_{Dl}$. Obviously there is no genetic reason for a correlation between the size of the litters in which the grandsires, *S*, and the grandams, *D*, were farrowed. But columns 1–3 of Table II of Wentworth and Aubel actually give:

$$r_{SD} = .121 \pm .022,$$

a value quite as large as those recorded above.

Such a correlation might arise (*a*) through the existences in the pens of different breeders of strains slightly differentiated with respect to fertility, (*b*) through differences in the conditions in which different breeders maintained their pens, providing such conditions affect litter size, or (*c*) through actual dishonesty of certain breeders in reporting the size of litters for herd-book publication.

Such differentiation, if it exists, would also account in part at least for the correlations hitherto regarded as due to inheritance. The whole problem is evidently one of great complexity and requiring far more detailed investigation than it has yet received.

The problem of the inheritance of the production of twins in sheep which has been studied experimentally by Alexander Graham Bell for the past several years, has recently been investigated statistically by Rietz and Roberts.[32]

There seems to be unmistakable evidence of inheritance, or at least of ascendant influence,[33] upon descendant characteristics. This may be most clearly seen by comparing the average number per litter resulting from certain matings.

Thus for the parental relationship the results are:

When sire and dams are singles	1.3452 ± 0.0059
When sire is single and dam is twin	1.4171 ± 0.0067
When sire is twin and dam is single	1.3946 ± 0.0073
When sire is twin and dam is twin	1.4548 ± 0.0088
When either sire or dam is a triplet	1.6076 ± 0.0300
Mean of all offspring	1.3979 ± 0.0035

[32] Rietz, H. L. and E. Roberts, "Degree of Resemblance of Parents and Offspring with Reference to Birth as Twins for Registered Shropshire Sheep," *Jour. Agr. Res.*, 4: 479–510, 1915.

[33] In the case of slight relationships between parents or earlier ancestors and offspring there is always danger of attributing to heredity the influence of purely physiological factors.

Or for the dams and grandams:

When dams and grandams are singles	1.3446 ± 0.0057
When the dams are singles and grandams twins	1.3689 ± 0.0070
When the dams are twins and grandams are singles	1.4245 ± 0.0071
When the dams are twins and grandams are twins	1.4559 ± 0.0078
When either dam or grandam is a triplet	1.545 ± 0.037

Finally for the maternal grandams alone:

When the maternal grandams are singles	1.3784 ± 0.0045
When the maternal grandams are twins	1.4120 ± 0.0052
When the maternal grandams are triplets	1.556 ± 0.033

It is quite out of the question to review in any detail the thorough analysis of the numerous interrelationships deduced from the many thousands of records abstracted by the authors from the Shropshire record. Their data seem to be free from the possible objection raised against the swine records above, for the correlation between sire and dam, which may be deduced from their Table I, is only $r = .0058 \pm .0070$.

The intensity of correlation between the size of litter in which an individual is born and the size of the litter in which his sire or dam or grandsire or grandam was born is very low. The maximum relationships are in fact of the order $r = .08$.

In the parental relationships the correlation between the size of litter in which the sire was born and the size of the litter in which his offspring were born seems to be significant, as well as that between the size of the litter in which the dam was born and the number of the offspring. The mean number of offspring are:

When the sire was born single	1.3787 ± 0.0045
When the sire was a twin	1.4220 ± 0.0057
When the sire was a triplet	1.683 ± 0.045

Note the agreement of this result with that obtained by Wentworth and Aubel. An explanation on the basis of identical twins induced by the characteristics of the sperm, or of partial impotency in certain males, should be sought by those who have experimental facilities.

There seems to be a significant correlation between maternal

grandams and offspring, but it is impossible to assert any trustworthy correlation for the other grandparents.

INFLUENCE OF ENVIRONMENT ON FERTILITY

Marshall[34] while emphasizing the importance of the hereditary factor in multiple births in sheep, adduces evidences for the great importance of feeding as a factor in the production of twins and triplets. His figures certainly show great and consistent differences in the produce of flocks which have received different treatment at and preceding tupping time. Unfortunately differences in breed may, but do not necessarily, cast some doubt on the interpretation of the data. The problem which he has attempted to solve by the analysis of schedules received from flock masters certainly deserves experimental study. Such investigations have actually been begun by Evvard who in a first[35] and second[36] and third[37] report on experiments with swine has given the results of various feeding upon the vitality of the offspring. Discussion of the data as they are presented in these papers falls outside the scope of a biometric review. Such work is, however, of great importance at a period of science in which heredity as contrasted with environment is apt to be assumed to be an all-important factor. It is a pity that such experiments as these of Marshall and Evvard can not be carried out in close cooperation with experts on the physiology of nutrition, so that differences in rations might be arranged on a uniform scale.

J. ARTHUR HARRIS

ON A BARNACLE, CONCHODERMA VIRGATUM, ATTACHED TO A FISH, DIODON HYSTRIX[1]

A SPECIMEN of the "sea porcupine," *Diodon hystrix* Linn., seen swimming near the surface and secured with a dipnet, was

[34] Marshall, F. H. A., "Fertility in Scottish Sheep," *Trans. High. Agr. Soc. Scotland*, V, 20: 139–151, 1908.

[35] Evvard, J. M., "Nutrition as a Factor in Fetal Development," *Proc. Amer. Breed. Ass.*, 8: 549–560, 1912.

[36] Evvard, J. M., "Some Factors affecting Fetal Development," *Proc. Iowa Acad. Sci.*, 20: 325–330, 1913.

[37] Evvard, J. M., A. W. Dox and S. C. Guernsey, "The Effect of Calcium and Protein Fed Pregnant Swine upon the Size, Vigor, Bone, Coat and Condition of the Offspring," *Proc. Iowa Acad. Sci.*, 21: 269–278, pl. 31–35, 1914.

[1] Contributions from the Bermuda Biological Station for Research, No. 50.

found to have two living lepad barnacles attached to one of its erectile spines[2] upon the ventral surface two centimeters to the right anteriad of the anus. The *Diodon* was a small individual, 16 cm. long. It was kept under observation in the laboratory for several weeks.

According to a determination for which I am indebted to Mr. H. G. Coar, the barnacles belong to the species *Conchoderma virgatum* (Spengler), although varying "a trifle from Gruvel's type description, but not sufficiently to correspond to *Conchoderma hunteri* R. Owen, 1830, which the specimen approached slightly, nor to Leach's (1818) variety *chelonophilus* of *C. virgatum*." This species has not previously been recorded from the Bermuda area, though it is known over the Atlantic generally and (to judge from statements of fishermen) occurs here upon young turtles. *C. virgatum* has been found on *Mola*, ships' bottoms, and various other objects (Pilsbry, 1907, p. 99), but the present record is somewhat unusual.

Different semiparasitic lepads have quite various hosts, such as medusæ, antipatharians, the spines of echinoids, molluscs, crustaceans, sharks, teleosts, turtles, the tail feathers of sea birds, whales, and so forth (Pilsbry, 1907; 1910). Those occurring on fishes seem, naturally, to affix themselves to some hard part, for example, the head, as in the case of *Tylosurus* (Sumner, Osburn, and Cole, 1913, p. 647). Jordan (1905, p. 341, fig. 226) figures a flying fish with conchodermas attached to a *Penella* growing on the fish, a condition of double parasitism which has been described for Xiphias. In the present instance, the larger of the two conchoderma individuals (20 mm. long) was found to have its peduncle completely surrounding the spine to which it had become fixed. The second individual was much smaller (4 mm. long) and attached to the peduncle of the first. Both specimens were so oriented that the opening between the valves was directed toward the head of the fish. The skin of the fish about the base of the spine was inflamed, and the muscles which normally control its elevation for defensive purposes had apparently degenerated. When it was attempted to preserve the *Diodon*,

[2] The figure of *Diodon hystrix*, which is used in current ichthyological handbooks, represents the animal in a semipuffed-up condition and with the frontal spines erected. Alive, the fish has a quite different aspect, all the spines being flattened down to the skin unless the creature is much disturbed. When preserved in formalin it assumes the appearance depicted in the handbooks.

the spine bearing the conchodermas became detached in the course of the animal's self-inflation. It is probable, therefore, that the spine would soon have been shed under natural circumstances.

Several features of the behavior of these conchodermas are of interest in comparison with those of other barnacles. Some years ago it was reported by Pouchet et Joubert (1876) that cirripedia attached to rocks reacted to shading, while those attached to floating objects did not; their inference being that to the stationary barnacles a shadow signified danger, whereas, to those borne about at the surface of the water, a fluctuating illumination was the normal state of affairs. This observation has been regarded as an instance of adaptation comparable with that of Hargitt (1909) on the gradual loss of reaction to shading when serpulids are maintained in the laboratory.

The specimens of *Conchoderma* attached to *Diodon* did not react to shadows under any of a number of experimental conditions. They seem, therefore, to be in agreement with the observation of Pouchet et Joubert. But tests upon lepads found upon floating timbers and upon *Ascophyllum* showed that *Lepas anserifera* and *L. pectinata* do respond to shading by retracting the legs and approximating the valves. From a number of tests it appeared that neither the legs nor valves are sensitive to shading, but that the shadow must affect some part of the body within the shell suggesting that the persisting nauplius eye is the organ involved. The extent of the response varies with the degree to which the appendages have been extruded: when just being extruded, they react by complete retraction; when fully extruded, by a partial retraction; after being fully extruded for one or two minutes, they react to shading quite promptly and completely. After completion of a response there must usually elapse from two to four minutes before another reaction can be secured.

It seems to me, then, that the supposed adaptation of floating barnacles is not of the nature which has been supposed. Whether the non-reaction of *Conchoderma* to shading is properly to be considered a direct adaptation is therefore questionable. The host of these particular specimens is not a surface fish, and the absence of sensitivity to shading may be due to their deep habitat. Direct sunlight inhibited the rhythmic movements of the conchodermas, and they were much more active at night than in diffuse laboratory light.

The statement is occasionally met with that in barnacles attached to a free-swimming animal the feathery feet are merely thrust out, not waved about as in the rock barnacles, which must create food- and respiratory-currents for themselves. Now, it was observed that when the *Diodon* bearing the conchodermas was actively swimming, the legs of the lepads remained extended for as much as four to five minutes; whereas, when the fish remained stationary, they were alternately extended and retracted about seven times every minute (at 18° C.), the extension in the latter case being not so great as when their host was moving. *Lepas anserifera* and *L. pectinata* were then tested as to their behavior in currents, with this result: when the wood to which they were attached was stationary, the rhythmic contraction of the appendages was continuous, but if a gentle stream of water from a supply jet was allowed to flow past them impinging on the anterior (concave) edges of the legs, they remained extended for as long as ten minutes, and were spread farther apart than in the absence of the current. This was not due to any merely mechanical effect of the water stream, as the feet could at any time be caused to contract at a touch. A water stream, striking the posterior (convex) edges of the legs, led to contraction and subsequent limited extrusion of these appendages. A more correct interpretation of the phenomenon described in floating barnacles seems to be, therefore, that when the concave side of the appendages is stimulated by a water current, the animal responds by pushing out its legs further than is usual in the absence of currents, while their rhythmic contraction is inhibited. It should be noted that the two specimens of *Conchoderma* observed were so oriented on the *Diodon* as to receive the full benefit of currents derived from its forward swimming; and further, that this fish is not a vigorous swimmer, so that the currents in question are by no means rapid, but rather such as could be efficiently strained by the barnacles.

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THE EVOLUTIONARY SIGNIFICANCE OF THE OSMOTIC PRESSURE OF THE BLOOD[1]

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The facts of comparative anatomy, embryology and paleontology form the tripod of evidence on which rests to a great degree the validity of the doctrine of evolution. Accepting the doctrine of evolution as a working hypothesis has resulted in clearing up puzzling problems in the above named departments of biological inquiry. At the present time, more attention is being paid to physiological than to morphological problems. In physiology, the great emphasis is placed on mammalian problems with especial reference to man himself. Now if the mammals are the product of a long process of evolution from simple ancestors, it follows that not only has there been a morphological evolution, but also the present complicated functions of the higher animals have evolved from the simpler processes of primitive ancestral forms. In order to understand the significance of particular physiological facts, we must therefore view the matter in the light of evolution. It is not essential that all needful evidence be at hand to make perfectly clear the significance of the higher physiological activity. Indeed, it is well worth while at times to state clearly any of our problems in the evolutionary form and arrange the evidence accordingly.

[1] Read by title before The American Society of Naturalists, Philadelphia, Dec., 1914.

In this way we become aware of the need for information to clear up the question which inevitably arises.

It is commonly known that the blood and body fluids of animals possess a certain osmotic pressure. Life processes are constantly dependent on the passage of materials in and out of cells and differences in the osmotic pressure of substances within and without the cell are held to be one cause of this mutual movement. Variations in the osmotic pressure of the blood and body fluids of animals are not so generally known. In the case of severe hemorrhage it is a common practice to replace the lost blood by a physiological salt solution which has the same osmotic pressure as that of the blood. Formerly a 0.7 per cent. saline solution was used. This is isotonic with amphibian blood. The reason for this was that the fact was first discovered in a study of frog's blood. The saline solution (based on amphibian studies) of the physiological laboratories was considered proper for use in hospitals as well. Later it was found that a 0.9 per cent. saline solution represents more nearly the composition of human blood and this solution is in use at present.

But why does human blood have an osmotic pressure equivalent to that of a 0.9 per cent. saline solution? In order to answer this question we must examine all available data as to the osmotic pressure of the blood and body fluids of animals in general. When this is done it appears in many cases at least as though the osmotic pressure of the blood and body fluids were merely a direct adaptation to the environment. But in other cases this is not so clearly apparent, in fact the osmotic pressure possessed by certain forms shows no evident adaptation to the environment at all. The terrestrial vertebrates illustrate this last condition. It is only when we view the entire question from the standpoint of evolution that the main features of the puzzle become apparent.

It might be well to explain at this point the meaning of osmotic pressure. One gram molecule of hydrogen gas at atmospheric pressure occupies 22.4 liters space, and to confine this gas in a space of one liter would require a

pressure of 22.4 atmospheres. A gram molecule of any other gas under the same conditions has the same pressure. Van't Hoff in his theory of solutions established the fact that a substance in solution behaves as a gas occupying the same volume as the solution and the laws which solutions obey are analogous to those which are followed by gases. Therefore a gram-mol of a substance dissolved in a liter of pure water would have the same pressure as a gram-mol of gas, *i. e.*, 22.4 atmospheres. This pressure property of dissolved substances is called osmotic pressure. Since the blood and body fluids contain salts and other substances in solution, these fluids therefore have a certain osmotic pressure. It is well known that a salt solution has a lower freezing point than that of pure (distilled) water. The difference is proportional to the difference in concentration. Since the osmotic pressure depends on the concentration, it follows that the amount of the depression of the freezing point of the solution below that of distilled water is a measure of the osmotic pressure. The osmotic pressure stated in atmospheres can be readily obtained from the "Δ" or depression of the freezing point by the use of the following formula. Osmotic pressure in atmospheres = $(\Delta \times 22.4)/1.85$.

The blood of a vertebrate serves two double purposes. It carries oxygen to tissues and carbon dioxide away. This is its respiratory function. It also carries nutrients to tissues and wastes of metabolism from tissues. We might call this the nutrient function. But the blood of the earthworm is mainly a respiratory fluid. The body cavity is filled with foods absorbed directly from the intestine and distributed by the peristaltic movements of the body to the various tissues. In insects the air is carried directly to the tissues through tracheæ while a so-called heart lying on the dorsal side of the intestine and open at its anterior and posterior ends aids in churning and distribution of food absorbed into the body cavity from the intestine. The indefiniteness of the term "blood" is at once apparent. Most persons in using this

TABLE I

SHOWING THE FREEZING POINTS (Δ) OF THE BLOOD OF ANIMALS

Species	Δ Blood	Δ Water	Locality	Observer
I. Coelenterate:				
1. *Alcyonium*	2.195	2.29	Naples	Bottazzi
II. Echinodermata:				
2. *Asteropecten*	2.312	2.29	Naples	Bottazzi
3. *Asterias*	2.295	2.29	"	"
4. *Holothuria*	2.315	2.29	"	"
III. Annelida:				
5. *Sipunculus*	2.31	2.29	Naples	Bottazzi
IV. Arthropoda:				
6. *Homarus vulgaris*	2.292	2.29	Naples	Bottazzi
7. *Maja squinada*	2.36	2.29	"	"
8. *Maja verrucosa*	2.13	2.29	"	Fredericq
9. *Homarus americ.*	1.82	1.80	Woods Hole	Garrey
10. " "	1.78	1.76	St. Andrews	Macallum
11. *Limulus*	1.90	1.82	Woods Hole	Garrey
12. "	2.04	?	?	Macallum
13. *Astacus*	0.80	0.03	....	Fredericq
14. *Barbus*	0.475	0.03	....	"
V. Mollusca:				
15. *Aplysia*	2.31	2.29	Naples	Bottazzi
16. *Octopus*	2.24	2.29	"	"
VI. Cyclostomata:				
17. *Polistrotema*	1.966	1.924	Monterey	Greene
VII. Elasmobranchii:				
18. *Mustelus vulg.*	2.36	2.29	Naples	Mosso
19. *Trygon viol.*	2.44	2.29	"	"
20. " "	2.378	2.29	"	Bottazzi
21. *Mustelus laev.*	2.36	2.29	"	"
22. *Scyllium stell.*	2.31	2.29	"	"
23. *Torpedo ocell.*	2.351	2.29	"	"
24. *Torp. marmorata*	2.292	2.29	"	"
25. *Squatina angelus*	2.28	2.29	"	"
26. *Acanthias vulg.*	1.90	1.91	North Sea	Dakin
27. *Raia clavata*	1.90	1.91	" "	"
28. *Carcharias lit.*	2.03	1.82	Woods Hole	Garrey
29. *Mustelus canis*	1.88	1.82	" "	"
30. *Mustelus canis*	1.869[2]	1.81	" "	Scott
31. *Squalus acanthias*	1.84	1.81	" "	"
32. " "	1.70	1.42	New York	"
VIII. Pisces:				
33. *Acipenser sturio*	0.76	2.00	Arcachon	Rodier
34. *Charax*	1.040	2.29	Naples	Mosso
35. *Serranus*	1.035	2.29	"	"
36. *Conger vulg.*	1.120	2.29	"	Bottazzi
37. *Deutex vulgaris*	1.022	2.29	"	"
38. *Oncorhynchus*	0.762	1.924	Monterey	Greene
39. *Pleuronectes fles.*	0.883	1.91	North Sea	Dakin
40. *Pleuronectes plat.*	0.71	1.91	" "	"
41. *Lophius*	0.80	2.00	Arcachon	Rodier
42. *Lump sucker*	0.648	1.90	North Sea	Dakin
43. *Gadus mor.*	0.72 (0.64)	1.80	Baltic	Dekhuyzen
44. *Pleuronectes*	0.681	1.80	"	"
45. *Conger vulg.*	0.74	1.80	"	"
46. *Cottus scorp.*	0.941	....	Amsterdam	"
47. " "	1.178	....	Helder	"

[2] Mean Δ of eighty specimens.

Species	Δ Blood	Δ Water	Locality	Observer
VIII. Pisces:				
48. *Gadus angel.*	0.767	1.80	Baltic	"
49. *Gadus virens*	0.76	1.80	"	"
50. *Gadus merl.*	0.86	1.80	"	"
51. *Molva vulg.*	0.716	1.80	"	"
52. *Molva byrkel*	0.86	1.80	"	"
53. *Motella tric.*	0.605	1.80	"	"
54. *Hippoglossus*	0.671	1.80	"	"
55. *Pleuronectes pl.*	0.672	1.80	"	"
56. *Pleuron. micro*	0.681	1.80	"	"
57. *Labrus bergylta*	0.694	1.80	"	"
58. *Labrus mixtus*	0.681	1.80	"	"
59. *Conger vulg.*	0.696	1.80	"	"
60. *Salmo trutta*	0.785	1.80	"	"
61. *Labrax lupus*	0.72	1.80	"	"
62. *Trigla hirundo*	0.669	1.80	"	"
63. *Anarrichas*	0.665	1.80	"	"
64. *Agonus cataphr.*	1.095	....	Helder	"
65. *Zoarces*	1.30	....	"	"
66. *Tautoga onitis*	0.86	1.82	Woods Hole	Garrey
67. " "	0.70	1.42	New York	Scott
68. *Cyonoscion*	0.792	1.82	Woods Hole	Garrey
69. *Conger eel*	0.82	1.82	" "	"
70. *Anguilla*	0.90	1.82	" "	"
71. "	0.635	1.91	North Sea	Dakin
72. *Scup*	0.75	1.82	Woods Hole	Scott
73. *Morone am.*	0.735	1.82	" "	"
74. *Oncorhyncus*	0.628	0.03	Fresh water	Greene
75. *Morone am.*	0.571	0.03	" "	(Scott)
76. *Anguilla*	0.57	0.03	" "	Dakin
77. *Pleuronectes*	0.68	0.03	" "	"
78. *Perca*	0.507	0.03	" "	Dekhuyzen
79. *Esox lucius*	0.519	0.03	" "	"
80. *Salmo fario*	0.567	0.03	" "	"
81. *Abramis blicca*	0.497	0.03	" "	"
82. *Cyprinus carpoo*	0.527	0.03	" "	"
83. *Tinca vulgaris*	0.519	0.03	" "	"
84. *Leuniscus eryth.*	0.495	0.03	" "	"
85. *Erythrinus*	0.577	0.03	" "	"
86. *Abramis brama*	0.51	0.03	" "	Dakin
87. *Cyprinus carpio*	0.487	0.03	" "	"
IX. Amphibia:				
88. *Rana escul.*	0.563	....	....	Bottazzi
89. *Bufo viridis*	0.761	....	....	Bottazzi & Duceeschi
90. *Bufo vulgaris*	0.445	....	....	Bottazzi
X. Reptilia:				
91. *Thallassochelys*	0.61	2.29	Naples	Mosso
92. *Emys europa*	0.463	2.29	"	Bottazzi & Duceeschi
93. " "	0.440	2.29	"	Bottazzi
XI. Aves:				
94. Capon	0.66	....	....	D'Errico
95. Turkey	0.75	....	....	"
96. *Gallus bank.*	0.623	....	....	Bottazzi
XII. Mammalia:				
97. *Delphinus phocœna*	0.74	1.90	....	....
98. Horse	0.58	....	....	Findlay
99. "	0.565	....	....	Winter

Species	Δ Blood	Δ Water	Locality	Observer
XII. Mammalia:				
100. Ox	0.601	....	....	Findlay
101. "	0.55	....	....	Winter
102. Pig	0.625	....	....	Findlay
103. "	0.55	....	....	Winter
104. Dog	0.599	....	....	Findlay
105. "	0.565	....	....	Winter
106. Rabbit	0.578	....	....	Findlay
107. "	0.57	....	....	Winter
108. "	0.564	....	....	Bottazzi & Ducceschi
109. Sheep	0.55	....	....	Winter
110. Cat	0.615	....	....	Findlay
111. MAN	0.560	....	....	"

term think of the fluid circulating in the blood vessels of a vertebrate. The term body fluid is also ambiguous. In an invertebrate it has reference to that part which we call the blood of a vertebrate. In the vertebrate we usually think of the secretion of serous membranes as "body fluid." After all, the subject of discussion in this paper is the fluid by which food is carried to tissues and wastes carried away. Having thus defined the use of the terms, let us examine the osmotic pressures of the blood of various animals.

Table I, which follows, shows one hundred and eleven determinations of the osmotic pressure of the blood of representatives of nearly every animal phylum. Many of these determinations are averages. Some of the forms are wholly terrestrial, some live in fresh water, some in either fresh or seawater, some live wholly in the sea. Considerable variation in the osmotic pressure of the blood is shown.

Of the marine forms given some are found in the Mediterranean, while others for the most part occur in the ocean or in protected waters connected with it. There is great variation in the osmotic pressure of the blood of forms living exclusively in the Mediterranean. Great variation is shown in the case of those living in the ocean. In some cases in each environment, complete harmony with or rather isotonicity with the environment is apparent. In other cases this is not at all evident. For

example, the average Δ of twelve species of invertebrates from the Mediterranean is 2.281°, while the average Δ of the water in which they live is 2.29°. A simple case of adaptation is thus evident. But the bony fishes, teleosts, tell a different story.

It is worth while to contrast the osmotic pressure of the blood with that of the external medium. To do this we will break up all the forms into groups not according to the environment alone, but also according to relationship. If we should be guided by environment alone, the result would be a confused tangle. Table II shows the average Δ of these groups selected not only on the basis of relationship but also taking into partial consideration the environment.

"Δ," Blood,	12 Invertebrates,	Mediterranean	= 2.28°	—Water = 2.29°
"Δ," Blood,	4 Invertebrates,	Ocean, bays	= 1.82	—Water = 1.79
"Δ," Blood,	3 Invertebrates,	Fresh water	= 0.592	—Water = 0.03
"Δ," Blood,	1 Cyclostome,	Ocean, bay	= 1.966	—Water = 1.924
"Δ," Blood,	8 Elasmobranchs,	Mediterranean	= 2.346	—Water = 2.29
"Δ," Blood,	6 Elasmobranchs,	Ocean, bays	= 1.902	—Water = 1.85
"Δ," Blood,	4 Teleosts,	Mediterranean	= 1.054	—Water = 2.29
"Δ," Blood,	32 Teleosts,	Ocean, etc.	= 0.744	—Water = 1.82
"Δ," Blood,	13 Teleosts,	Fresh water	= 0.545	—Water = 0.03
"Δ," Blood,	4 Amphibia,	Fresh water	= 0.551	—
"Δ," Blood,	6 Reptilia,	....	= 0.56	—
"Δ," Blood,	3 Aves,	....	= 0.67	—
"Δ," Blood,	8 Mammals,	....	= 0.577	—

From this table it is evident that the blood of the marine invertebrate is isotonic with the water in which it lives, whether this be the Mediterranean or the ocean. As stated above, it appears to be a simple case of adaptation. But in the other cases the relation is not so simple. If we compare the osmotic pressure of the marine teleosts, fresh-water teleosts and the amphibia, etc., with the osmotic pressure of the external medium great differences are evident. And yet it can not be said but what all these forms are adapted to their environment. But it is not enough to make this statement, but to try to explain why such a relationship becomes possible. The isotonicity existing between the blood of marine invertebrates and

their environment has been discussed by Fredericq ('85–'04), Rodier ('99), Dakin ('08), Garrey ('05) and Bottazzi ('97–'06). Now it is held that evolution of life began in the sea. The single celled forms were completely surrounded by the sea and it is easily understood why the osmotic relations would remain primitive in case of these forms. In gastrula type animals, such as cœlenterata, practically all cells of the body are bathed directly by the sea and as far as we know these forms also are in osmotic equilibrium with sea water. Now with the appearance of mesoderm and a body cavity much of the body is removed from direct contact with the sea. But the complete equilibrium remains. As Quinton ('00) says, the marine invertebrate, though anatomically independent of the sea in many of its organs, yet it is still physiologically open to the sea which in an osmotic sense still ebbs and flows throughout its body.

Protoplasm originating in the sea was built up with certain relationships with sea water, which relationships are still maintained throughout all marine invertebrates. May not the sparsity of fresh-water porifera and cœlenterates and the comparative failure of fresh-water algæ be due to the difficulty of maintaining the integrity of protoplasm when all cells of these forms are so freely bathed by fresh water, the osmotic pressure of which is nearly zero?

Next above the marine invertebrates is a single case of a cyclostome which is in osmotic equilibrium with the surrounding sea water. What the osmotic pressure of the blood of a cyclostome in fresh water is, we have no record. It should be noted here that cyclostomes are now regarded as degenerate fishes and on that account any evidence from these forms as to the higher course of evolution must be treated with care. In the next place we find that eight species of elasmobranchs from the Mediterranean and six from the ocean possess blood which is practically isotonic with the sea water outside. Apparently they do not differ from the marine invertebrates. But it is evident that the osmotic pressure of the blood is slightly

greater than that of the external medium. Furthermore, analysis shows that the osmotic pressure of elasmobranch blood is due to different substances from those which account for the osmotic pressure of the blood of marine invertebrates. Therefore the elasmobranchs belong to a second category. In the third group we will place the marine teleosts. The osmotic pressure of their blood is somewhat less than half that of the medium in which they live. We have the case of four species from the Mediterranean and thirty-two species from the ocean which show this. The osmotic measurements show a decided difference between the blood and the surrounding medium. A decided independence also. In the same group or possibly a fourth group we will place the fresh-water fishes and with these the amphibians, reptiles, birds and mammals. Thirteen species of fresh-water fishes possess blood with an osmotic pressure less than that possessed by the marine teleosts. Let us assume here that the fresh-water fishes were derived from marine ancestors. In becoming acclimated to fresh water, the blood suffered a decrease in its osmotic pressure. Whether this was in direct response to the great decrease in the osmotic pressure of the surrounding medium as compared with sea-water is problematical, but appears probable. The amphibians were derived from the fresh-water teleosts. Some of the amphibians still retain their aquatic habits and structures. They in all probability possess the osmotic pressure of fresh-water fishes. Other amphibia metamorphosed into terrestrial forms, taking with them the osmotic pressures of the blood possessed by their fish-like ancestors. Blood with the same osmotic pressure as that of the fresh-water fishes flows on through the amphibia to the reptilia and on to the birds and mammals. An examination of Table II shows the close similarity between the osmotic pressures of fresh-water fishes, amphibians, reptiles, birds and mammals. According to the above hypothesis, the order of evolution was I. Marine invertebrates, II. Elasmobranchs, III. Marine teleosts,

IV. Fresh-water teleosts, amphibians, reptiles, birds and mammals.

Let us examine each of these groups with regard to their osmotic independence of the external medium. That is, what is the effect of changes in the concentration of the external medium on the osmotic pressure of the blood of these groups.

First, the invertebrates. Let us recall Quinton's statement that marine invertebrates are still physiologically open to the sea. For when the concentration of the external medium is changed, it is found that a change in the osmotic pressure of the blood takes place. Fredericq ('85 and '04) stated that the change in one was followed by an equal change in the other. In a few hours the new equilibrium is established. If the time of sojourn in the modified sea water was small the equilibrium with it was not completely attained. Moreover, all invertebrates did not adapt themselves with the same rapidity to changes in the external medium. On the whole, provided the external change was not too great, it was followed in time by complete equilibrium between the osmotic pressure of the blood and that of the modified sea water. This was true in the case of sea water made dilute by addition of fresh water and sea water made more concentrated by the addition of salt. In other words, the organism possesses no structures which render it independent of the changes in the external medium. There are three structures concerned in these changes. First the integument, second, the intestinal wall and third the gill membranes. With the appearance of gills, the body integument apparently is the first structure to become impermeable. The intestinal wall is the first to show a selective action.

Second, the elasmobranchs. These had been placed by investigators with the marine invertebrates not only because their blood possessed the same osmotic pressure as the external medium, but it was thought that when the external medium was changed, the same changes occurred in the blood of the elasmobranch. I made extensive ex-

periments to test this ('13) and found that when a change was made in the external medium, though considerable change took place in the blood of the dogfish, yet it was considerably less than the external change. In fact it appeared as though the change in the blood was roughly proportional to the change in the external medium (p. 20, Scott, '13). The condition was so marked as to show clearly that the elasmobranch belonged in a category differing from that of the marine invertebrate.

Third, the marine teleost. Much emphasis has been placed upon the claim that these forms are absolutely independent of changes in the external medium. With this claim, I must differ. The following evidence is the basis of this difference of opinion. In the first place Tables I and II show that the blood of teleosts from the Mediterranean has a higher osmotic pressure than that of blood of teleosts from the ocean. There is a corresponding though greater difference in the osmotic pressure of the water. Dakin '08 in a trip from Kiel to Helgoland found that the osmotic pressure of the sea water increased 74 per cent. and that the osmotic pressure of the blood of the plaice showed an increase of 20 per cent. The cod did not show so great a difference, being but 4 per cent.[3] Garrey '05 reported Δ of the blood of the tautog at Woods Hole to be 0.86° while at the New York Aquarium, where the harbor water is much more dilute than at Woods Hole, I found the Δ of tautog blood to be about 0.70°. Therefore it would appear that even blood of the marine teleost is somewhat modified by changes in the external medium. And yet practical independence has been achieved. This is evident from the fact that the marine teleost lives in a medium which has an osmotic pressure over twice as great as that of the blood of the fish.

Macallum ('10) has explained the peculiar osmotic pressure of the blood of marine teleosts as due to their origin from fresh-water teleosts. This is based on morpholog-

[3] On the other hand Dekhuyzen, '05, found a difference of 20 per cent. in the osmotic pressure of cod blood according to the locality from which the fish was taken.

ical evidence of the evolution of the true teleosts from ganoid ancestors from the elasmobranchs through forms similar to the sturgeons and the bow-fins. I doubt very much, however, whether ichthyologists would wish to conclude on this basis that all marine teleosts had their origin from fresh-water forms. In fact certain paleontologists trace the evolution of certain fresh-water teleosts from ancestral marine teleosts. The sea is the home of the preponderating fish population. Here the class of Pisces has found its greatest opportunities for range of movements to escape enemies, in search of food or place of breeding.

Facts concerning the osmotic pressure of the blood of anadromous fishes throw light as to the possible if not probable origin of fresh-water forms. Greene ('04) determined the osmotic pressure of the chinook salmon in Monterey Bay to be 0.76°. On the spawning grounds in fresh water its blood had a Δ of 0.628°, a decrease of 17.6 per cent. Flatfish are known to be somewhat anadromous. Dakin ('08) found the Δ of the flounder, *Pleuronectes flesus,* in the North Sea to be 0.83°, while in the River Elbe in fresh water its blood had a Δ of 0.68°, a decrease of 18 per cent. The same author found that the blood of the eel, *Anguilla,* in fresh water had a Δ of 0.57°, quite similar to that of fresh water fishes. After a day in sea water another specimen had blood with a Δ of 0.745°. Eels taken from seawater had blood with a Δ of 0.634°. Eels from seawater placed in fresh water for three days possessed blood with a Δ of 0.582°, practically the same as for fresh-water forms. At Woods Hole, ignorant of this work of Dakin's, I made observations on the Δ of the blood of the white perch, *Morone americana.* This form can live equally well in salt or fresh water. Taken from the slightly brackish waters of Tashmoo Pond, Marthas Vineyard, Massachusetts, the blood showed a Δ of 0.635°. The upper end of this pond is the source of drinking water for Oak Bluffs. A number of perch were placed in running tap water for a day, when the blood showed a

Δ of 0.571°, similar to the fresh-water fishes. Others of this lot were placed in sea water for two days, when the Δ of their blood was 0.766°. Others taken directly from the Eel Pond (sea water) showed a Δ of 0.735°. The result is similar to Dakin's. On the whole the conclusion seems justified that anadromous fishes are able to adapt themselves to a degree to the great changes in the osmotic pressures of the external medium, which they meet in passing from salt to fresh water or vice versa by a slight corresponding change in the osmotic pressure of the blood.

It is commonly known that sturgeons are anadromous. For some reason the elasmobranch has been shut out of fresh water. There is but one elasmobranch known to inhabit fresh water, *Carcharias nicaraguensis* of certain lakes in Nicaragua. Although the integument of the shark is impermeable, yet I have found the gills to be still permeable to salts (Scott & Denis, '13). The ganoids derived from elasmobranchs ventured up fresh-water streams. They returned to the sea. Rodier ('99) states the Δ of the blood of *Acipenser sturio* to be 0.76°, which places it in the same group as the marine teleosts. What the Δ is in fresh water is not known. The modern sturgeon is a long way from the modern shark. Nevertheless it is conceivable that the ancestral ganoids tried fresh-water conditions. Is it not possible that these conditions, fresh water and food found in fresh water had some influence on the change in structure. During all subsequent periods when evolutionary changes were taking place some forms went back and forth from sea to fresh water. Some forms remained in fresh water. During this period of experimentation, impermeable membranes were built up. In the meantime the blood had become modified, due to the temporary sojourn in fresh water. The osmotic pressure was reduced; the membranes once made practically impermeable remained so, and when those forms returned to the sea and remained there they retained *almost* unmodified the osmotic pres-

sures they had acquired during their fresh-water experience. We can thus speculate that in some such way the present osmotic pressures of the blood of marine and fresh-water teleosts were acquired. Whatever may be the case with the marine and fresh-water teleosts, it is more clearly indicated that the osmotic pressure of the blood of terrestrial forms is derived from fishes which lived in fresh water. The present day anadromous fishes constitute all that remains of a movement which at one time was far more general.

The chemical composition of the blood throws further light on the question. The osmotic pressure is due to substances dissolved in the blood. These are chiefly salts. Quinton ('00) states that sodium chloride represents from 85 per cent. to 90 per cent. of all the dissolved salts of the blood. The sodium chloride content can be ascertained from a study of the chlorides which are easily determined. Let us ascertain the changes in the sodium chloride content of the blood of the forms under discussion. In the first place what is the total salt content of sea water. According to Bottazzi ('97) the total salt content of water from the Mediterranean is 3.78 per cent. The water of the ocean contains about 3.22 per cent. salts. Of course there is some variation. The percentage of salts in fresh water is very small, 0.05 per cent. (Sumner, '05). What is the percentage of salt of the blood of forms living in the sea? Quinton ('00) made forty-nine determinations of the sodium chloride content of the hemolymph of ten species of marine invertebrates belonging to five different groups and found that these averaged 3.24 per cent. He made 26 determinations of the sodium chloride content of the sea water in which these forms lived, and found that it was about 3.31 per cent. According to these researches of Quinton, the blood of the marine invertebrate contains about the same percentage of salts as the water in which they live. Moreover, it follows that the osmotic pressure of the blood is determined almost wholly by the salts of the blood and not by any organic solutes. It was because

of this relationship that Quinton felt justified in making the statement that the marine invertebrate while anatomically closed to the external medium, is yet physiologically open to it. That functionally speaking the marine invertebrate is still freely exposed to the sea without, which still practically ebbs and flows through its body.

Macallum ('10) says:

> In *Limulus,* the amount of total salts in the blood, 2.982 per cent., approaches that of the sea water,—which may be found along the Atlantic coast. At St. Andrews, New Brunswick, the total salts of the seawater collected in April were 2.417 per cent., but in sea water collected in August, 3.165 per cent. In the blood of the lobster, the total salts as ascertained were 2.852 per cent., which is between the two concentrations given above for the salinity of the sea water at St. Andrews where the lobsters from which the blood was taken were obtained. The blood of *Limulus* is but slightly modified sea water. It would appear as if the sodium chloride of sea water passes freely into the blood of the lobster till the sodium chloride concentration in both is approximately balanced.

This agrees entirely with the work of Quinton. For some reason, the marine invertebrate has not been able to keep the sea out. One asks why the question of the permeability of membranes of fishes to salts is of such interest to the comparative physiologist? One answer is that impermeability represents independence of the sea the osmotic pressure of which differs so much from that of fish blood. And this independence is not to be found among the marine invertebrates.

As shown above, elasmobranch blood possesses the same osmotic pressure as that of the marine invertebrate and that of the sea without. But analysis shows that the osmotic pressure of elasmobranch blood is due to entirely different causes. For example, what is the salt content of elasmobranch blood? It should contain about 3.22 per cent. salts in order that its total osmotic pressure be due to salts. But Rodier ('99) found that the blood of elasmobranchs did not contain over 1.7 per cent. sodium chloride. Dakin ('08) found the blood of the dogfish to contain but 1.45 per cent. sodium chloride. My analysis of the blood of another species, *Mustelus,* at Woods Hole

showed 1.424 per cent. sodium chloride. Fredericq ('04) found the blood of *Scyllium* to contain but 1.71 per cent. salts, while Macallum ('10) found the blood of the dogfish, *Acanthias vulgaris,* contained 1.7739 per cent. sodium chloride. In other words the sodium chloride content of the blood of elasmobranchs will account for only about half of its total osmotic pressure. Evidently a great change has come about. "The difference between the Δ of the serum and that due to salts of the serum depends," as Macallum ('10) says,

"on urea and other organic solutes." Urea is present in large quantities in the blood of elasmobranchs.

Staedeler and Frerichs ('58) obtained as much as two ounces from the the liver of a single shark. In '90 von Schroeder found that *Scyllium,* another dogfish, contained blood with 2.6 per cent. urea. Rodier ('99) computed that one third the osmotic pressure of the blood of elasmobranchs was due to urea.

In '13, I found that *Mustelus* blood contained 1.55 per cent. urea. Macallum ('10) in *Acanthias vulgaris* found an average of 2.026 per cent. urea. Due to dissociation, the salts have twice the osmotic pressure, approximately, as the urea, although the urea and salts are present in about equal quantities. But the urea and salts are not sufficient to account for the osmotic pressure of the blood. The difference is due to the presence of ammonia salts, as Macallum found. For example, he found 0.1727 per cent. ammonia in the serum of the dogfish. This would fully account for the remaining percentage of the depression of the freezing point unaccounted for by the presence of the salts and urea. So that we see, that while superficially the elasmobranch resembles the marine invertebrate in the osmotic pressure of the blood, yet below the surface a marked change has taken place. Several observers had noted that the osmotic pressure was slightly greater than that of the sea water. This at least is another indication that the equilibrium is not like that existing between marine invertebrates and the sea. For some reason the elasmobranch has lost in salts. Their place has been taken by *nitrogenous* solutes. The con-

dition is lacking in the marine invertebrate. Some one has characterized the jellyfish as organized sea water. According to Macallum the blood of *Limulus* is but slightly modified sea water. The blood of the marine invertebrate has remained at the same low level so far as the presence of nitrogenous compounds is concerned. To what may we ascribe this new condition? Is it due to great proportion of nitrogenous food? To the particular kind of liver? To the great development of the muscular system? To a peculiar function of the kidney? Questions can at present be asked only. We lack information as to certain aspects of elasmobranch physiology.

However much the elasmobranch may have experimented in the matter of unique nitrogenous content of the blood, it is certain that this condition is lacking in the teleosts. And the lack there is carried over to the forms which developed further. For the osmotic pressure of the blood of teleosts is again determined almost wholly by the salts present. The salt content of the blood of marine teleosts is considerably less than that of elasmobranchs. Quinton ('00) found the blood of eight species of marine teleosts to contain 1.076 per cent. salts. Rodier ('99) found that the blood of the ganoid, *Acipenser sturio,* had a salt content varying from 0.643 per cent. to 0.979 per cent. The blood of *Lophius,* a strictly marine form, contained 1.164 per cent. salts. Hamburger states that teleost blood contains 0.936 per cent. salts, but whether these are fresh-water or marine species is not stated. Macallum ('10) found that the blood of the cod, *Gadus callarias,* contained 1.2823 per cent., while that of the pollock, *Pollachius virens,* contained 1.2934 per cent. salts. It is evident, therefore, that the percentage of salts in the blood of the marine teleost has been decreased as compared with the total saline content of elasmobranch blood. Moreover, the osmotic pressure of the blood of the teleost is due almost wholly to the salts present. Macallum ('10) proved this. He found that the Δ of the salts of cod blood was 0.71°, while that of the entire blood was 0.765°.

There is a difference of but 0.055°. The Δ of the salts of the blood of the pollock was 0.737° while the Δ of the entire blood was 0.825, showing a difference of but 0.088°. In other words, almost the entire osmotic pressure of the blood of the teleost is due to the salts. The urea, ammonia or other organic solutes present must be very small and are represented by the difference above mentioned, namely 0.055° in the case of the cod and 0.098 in the case of the pollock. How different is this condition from that found in the elasmobranch where in one case noted by Macallum, and which is typical, the difference between the Δ of the saline contents of the blood and the entire blood was 0.961°, a difference as great as the average Δ of the marine teleost and as stated due to the relatively enormous amount of urea and other organic solutes in the blood of the dogfish. Again the question arises: What brought about this change between the composition of elasmobranch blood and that of the teleost? Was it due to the migrations to and from fresh water before certain species of teleosts took up their home permanently in the sea? And yet the marked difference between the two is not alone a difference in salt content. It is far more the absence from the blood of urea, ammonia and other organic solutes. Let us use Macallum's data as a basis for comparison. The blood of marine teleosts contains about 30 per cent. less salts than the blood of elasmobranchs but it contains 90 per cent. less organic solutes. The distinct loss therefor is in organic solutes. This therefore must have been a significant factor in the evolution of the higher form. Now what is the most apparent structural difference between the elasmobranchs and teleosts? It is of course that the skeleton of one consists of cartilage and the skeleton of the other is bone? It does not necessarily follow, however, that the power to build a bony skeleton depends on the absence of organic solutes from the blood, nor is there apparently any close connection between them.

The fresh-water fishes in all probability agree with the

marine teleosts in low percentage of organic solutes and this characteristic is maintained by all the higher forms. Dakin found that the blood of the plaice at Helgoland contained 0.92 per cent. salts, while at Kiel in brackish water it had a salt content of 0.85 per cent. Mosso ('90) stated that marine teleost blood had a higher salt content than that of fresh-water forms. Dakin ('08) found the blood of the eel in sea water to contain 0.605 per cent. salts, while in fresh water its saline content was 0.466 per cent. Quinton ('00) found that the blood of fresh-water teleosts contained 0.7 per cent. salts. Atwater ('91) found that the flesh of fresh-water teleosts contains less salt (15 per cent. less chlorine) than that of marine teleosts. Sumner ('05) obtained a similar result.

The anadromous fishes possess blood that is less saline in fresh water than in sea water. It is also true that strictly marine teleosts of the present day vary a little in the saline content of their blood when the salinity of the external medium changes. These facts indicate that the decreased salinity of the blood of fresh-water teleosts was brought about in response to the low saline content of the external medium. During the migrations that took place in the past when there were probably more anadromous fishes, this diminution in salts took place. Those forms that remained in fresh water retained the percentage of salts they acquired by their sojourn in fresh water. At the same time they built up membranes which maintain an equilibrium in spite of the differences in the osmotic pressure of the blood within and the fresh water without. Similar membranes were formed in case of the marine teleosts, which maintain an equilibrium with the sea water in spite of the fact that the osmotic pressure of sea water is over twice that of the teleost blood. The evidence at hand indicates that the last membranes to become practically impermeable to salts were the gill membranes. And yet though impermeable to salts they still are required to be permeable to gases.

Now the blood of amphibia contains about 0.7 per cent.

salts. This closely resembles that of fresh-water fishes. The blood of mammals contains a slight increase in its saline content. Bunge ('89) states that human blood serum contains about 0.84 per cent. to 0.86 per cent. salts. Macallum ('10) calculating from Abderhalden's analyses, concluded the total saline content of the blood of the dog amounted to 0.935 per cent., that of the cat to 0.933 per cent. and that of the sheep to 0.905 per cent. To quote from Macallum:

> In mammals, according to Abderhalden's analyses, there is an extraordinary similarity in the inorganic composition of the serum of the number of the forms taken and the ratios of the sodium, potassium, calcium, and magnesium are almost parallel with those in the Teleosts and Elasmobranchs.

Macallum had an opportunity to analyze the blood of "the whale common in the Pacific off the coast of British Columbia," and the parallelism between the inorganic constituents of its blood and that of the horse and pig was remarkable, thus bringing the whales very close to the Ungulates to which some anatomists relate them.

The above studies of the osmotic pressures of the blood, the change in the permeability of the protecting membranes and the inorganic and organic composition of the blood are understood only by viewing them from the standpoint of evolution. The increase in saline content of mammalian blood as compared with amphibian and fresh-water teleosts can be ascribed to the regulative action of the kidney. Most investigators give the impression that the osmotic pressure of the blood of animals is definite and fixed. This is not true. Findlay calls attention to the variation in the osmotic pressure of human blood at different times of day. For example, a distinct though slight rise (0.03°) is noted after meals. This question needs further study. My investigations showed that *Mustelus canis* can pass with entire safety through a range of 0.15° (+ and −) in its osmotic pressure. The range through which invertebrates can pass is much greater. The observations of Dekhuyzen ('05) and Dakin ('08) show that the range becomes limited in the case of

marine teleosts. The range is very much more restricted in fresh-water teleosts and higher forms. Protoplasm is an ever-changing substance. There is a constant ebb and flow. Protoplasm of the higher forms has evolved through long ages to a condition wherein it is associated with the same salts it was entirely surrounded by when it first began to be. The amounts have changed, but the proportions have remained quite constant and the kinds have remained the same as those in the sea. And that is why the surgeon must inject a 0.9 per cent. saline solution into the veins of his patient suffering from hemorrhage. And that is why human blood has a certain osmotic pressure. Macallum ascribes the first great reduction in salts which took place in the elasmobranch to be due to the kidney, whose primary function was not the elimination of the wastes of metabolism, but the regulation of the concentration and composition of the salts of the blood. The elasmobranch kidney is very inert and sluggish in the matter of the elimination of the organic wastes. The teleosts acquired the habit of still further keeping down the saline content while at the same time they eliminated the urea readily. However, I do not see that the process is necessarily limited to the kidneys alone. A thorough study of the elasmobranchs and teleosts is needed to throw light on this puzzle. I can see why the migratory habits of teleosts or teleost ancestors (ganoids) would account for reduction in salt content of the blood, but this throws no light on the reduction of salts in elasmobranch blood as compared with invertebrate blood. Nor does Macallum indicate any use the large amount of urea might serve. Balgioni ('06) found that salt solution alone led to stoppage of the elasmobranch ventricle in diastole. It increased diastolic tonus, while urea increased systolic tonus. The presence of the two in about equal amounts mutually neutralized each other and made the continuous rhythm of the heart possible. All we can say is that for the kind of protoplasm of which the elasmobranch heart is composed, the urea is a necessary constituent of the blood. Furthermore it does not appear to be necessary

for the teleost heart. At any rate we are aware that once we begin to question further, the necessity of further knowledge becomes evident. This paper can be brought to a close in no better way than by quoting a statement made by Claude Bernard ('65). We may accept it as one of the laws of evolution and conclude that inquiries concerning the osmotic pressures of the blood of animals amply prove its truth.

Chez tous les êtres vivants, le mileau intérieur, qui est un produit de l'organisme, conserve des rapports nécessaires d'éxchanges et d'équilibre avec le mileau cosmique extérieur; mais, à mesure que l'organisme devient plus parfait, le mileau organique se spécific et s'isole en quelque sorte de plus en plus du milieu ambiant.

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THE GENETIC BEHAVIOR OF MICE OF THE COLOR VARIETIES "BLACK-AND-TAN" AND "RED"

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Early in 1914 there were received at the Bussey Institution certain stocks of mice obtained from fanciers in England. Some preliminary studies of the mice were made by Professor Castle and Dr. Little. A more intensive study of one race, the black-eyed-white, was afterward made by Dr. Little and independently by Dr. Detlefsen. The remaining stocks were turned over to Mr. W. F. Whittier, who carried on experiments with them partly at the Bussey Institution, partly at the Massachusetts Agricultural College, recording some 2,500 offspring. After devising the color grading scale and the general methods followed in the later experiments, he relinquished the work to the present writer. Since that time about 2,000 young have been recorded, bringing the total to 4,500. All the work has been done under the advice and direction of Professor Castle.

The principal varieties which have been used in these experiments are known in England as "black-and-tans" and "reds." The genetic character of these mice was at the outset quite unknown, and in this paper it is proposed to give some account of their genetic behavior, and since they have proved to be forms of yellow mice, to assign to them and their derivatives places in a scheme of classification of the yellow varieties.

The black-and-tan race has presented throughout the more interesting problem. In appearance these mice are of an intense shiny black dorsally, with a belly superficially clear yellow. The belly hairs, however, are marked by having dull black bases, hidden by the longer and over-

lying yellow areas of the hairs. Yellow-ticked hairs are occasionally seen on flanks and head, encroaching on the black pigmented parts. This peculiarity increases somewhat with age, but never to such an extent as to make the body color predominantly yellow.

When bred *inter se,* they have been found invariably to be heterozygous, no homozygous black-and-tan having been discovered among a dozen individuals tested by suitable matings. As recessives they have given all-black mice more intense than any we have seen derived from other sources. Forty-two matings *inter se* of pure-bred black-and-tan parents produced 148 young, an average of 3.52 to a litter. Of these young 93 have been black-and-tan and 55 black, a ratio of 1.69 : 1. This approximates a 2 : 1 ratio more closely than the 3 : 1 ratio usually given by Mendelian heterozygotes. The black recessives breed true, and when mated to black-and-tans have produced equal numbers of black-and-tan and black young (18 : 18). The approximation of a 2 : 1 ratio in matings of black-and-tans *inter se* shows their gametic similarity to yellow mice whose unfixable nature was first shown by Cuénot ('03). Figures given by this author combined with those given by Castle and Little ('10), by Little ('10 and '11) and by Miss Durham ('11) total 2,673 young produced by yellow × yellow matings. Of these 1,783 were yellow and 890 non-yellow, a ratio of almost exactly 2 : 1.

The small average size of litters produced by black-and-tan parents mated *inter se* gives added evidence of their resemblance to yellow. Castle and Little ('10), in confirmation of Cuénot's observations, showed that yellow × yellow matings produced litters of smaller average size (4.71) than yellow × non-yellow (5.57), and following Cuénot they attributed the difference to absence of homozygous, yellow-yellow zygotes. The 2 : 1 ratio and the small-sized litters serve also to relate the black-and-tans with Castle's "sooties" and Miss Durham's "sables," both of which were shown to be heterozygous yellows carrying black as a recessive.

The reds, by their appearance, gave promise of being some form of yellow. The color, as the name implies, is orange-red dorsally, the belly being a lighter shade. Up to the age of three weeks the young mice are dusky yellow-red, the red apparently being obscured by a darker pigment. As they grow older they become progressively of a brighter and more intense reddish hue.

Genetically these mice behave much like black-and-tans. None has been found which has bred true, and the relation of reds to non-red recessives is in the same approximate ratio of 2:1. The recessives in this case are "chocolate," in color a deep, rich brown, showing an intensity comparable to that of the blacks derived from the black-and-tans. Thirty-one matings of red with red have produced a total of 136 young, of which 77 have been red, and 59 brown, a ratio of 1.30:1. The average size of these litters was 4.40. Eleven matings of red with brown produced 34 red and 31 brown young (equality expected), the average size of litters here being 5.90.

So far we have dealt only with the pure stocks, each of which is fairly uniform, although small fluctuations in density of pigmentation do occur. When, however, these two sorts are crossed with each other, yellow mice of various shades are obtained, which form two graded series, roughly parallel, one bearing black pigment and producing black recessives; the other bearing brown pigment and producing brown recessives. Classification in these two series is complicated by the fact that juvenile colors are not uniformly retained, but in some cases increase and in other cases decrease in intensity when the fur is moulted. All animals have therefore been assigned a numerical color-grade at the age of three weeks, this age having been determined as the time when the relation of yellow to black or brown pigment is most definitely visible; and although many animals have been re-graded at intervals throughout life, each has been designated by his original grade.

The cross of black-and-tan with red produced in F_1 two

classes of young. (1) One of these may be described as a black-and-tan in which the black pigmentation is lessened in amount and intensity, this decrease being attended by an increased development of yellow pigmentation. This class closely resembles the variety known as sable. (2) The other class of young consisted of blacks, which also were less intensely pigmented than the recessives produced by pure-bred black-and-tans mated *inter se.* It was found convenient in classifying the young of later generations to recognize six arbitrary grades of blackness of which yellow (showing no black pigment) forms grade 1, and black-and-tan grade 6. On this scale the mean of the F_1 "sable" young was close to 3.5, the intermediate point between yellow and black-and-tan. The distribution can be plotted by translating the descriptive terms in Mr. Whittier's notes into terms of numerical grades, as follows:

Grade	3,	4,	5,	6
Frequency	9,	0,	6,	1

These descriptive notes were made before the grading scale had been adopted, and it is quite probable that no real discontinuity in the variation occurred as would be suggested by entire absence of animals of grade 4. No such discontinuity is found in the work done since the grading scale was adopted.

The F_1 black young were mated *inter se* and back-crossed with browns to test their gametic composition. When mated *inter se* they gave 28 black and 11 brown young, nearly a 3:1 ratio (29:10). Back-crossed with browns they gave 37 blacks and 33 brown young, nearly a 1:1 ratio (35:35). F_1 blacks apparently, then, were simple heterozygotes, not differing from ordinary heterozygotes produced by crossing homozygous black with homozygous brown.

Thirteen of the F_1 sables were tested by mating with browns. One hundred and thirty-three young resulted, of which 70 were yellows of various shades and 63 non-yellows. Of this latter group 32 were black and 21 were

brown, equality being expected. The yellows also may be divided into two groups, in one of which the eyes and fur contain black pigment, while in the other the corresponding parts contain brown pigment. In both of these yellow groups the amount of black or brown pigment varied. Again translating Mr. Whittier's descriptions into terms of the numerical scale, we have the following distribution:

	Grade...	1,	2,	3,	4,	5,	6,	Total
(1) The black series—Frequency...		0,	4,	8,	3,	8,	2 (?),	25
(2) The brown series—Frequency..		13,	1,	4,	16,	1,	2,	37

It was frequently found to be impossible to determine by inspection alone whether a particular yellow animal belonged to the black or the brown series, because yellow fur containing a small amount of black pigment closely resembles that which contains a considerable amount of brown pigment. Consequently these back-cross young (produced by an F_1 sable mated with brown) had to be tested themselves, either by *inter se* matings or by crossing with browns. The classification of the back-cross young in the above tables is based partially on breeding tests and in the cases where these were lacking classification is based on inspection at the age of three weeks. It is uncertain whether any individuals were obtained from the F_1 sable × brown cross which showed the full intensity of pure-bred black-and-tans (grade 6), although two animals are recorded in the notes as black-and-tan without qualifying terms.

As a result of back-crossing with browns the F_1 sables (out of black-and-tan × red) and testing the young produced by crossing them with browns, two graded series of yellow mice may be recognized as follows.

Black Series			Brown Series		
Grade	Designation	Producing as Recessives	Grade	Designation	Producing as Recessives
6	Black-and-tan	Black	6	Brown-and-tan	Brown
3–5	Black-sable	Black	3–5	Brown-sable	Brown
2	Sooty yellow	Black	2	Red	Brown
1	Yellow	Black	1	Yellow	Brown

The brown-and-tan and brown-sable varieties are new. They resemble black-and-tan and black-sables, respectively, in which all black pigment in the fur has been replaced by brown pigment. The parallelism between the two series is strongest at top and bottom; red has no exact counterpart in the black series, since its yellow is more intense than that of sable. All members of the two series when crossed *inter se* fluctuate about their parental mean grade. The greatest fluctuations are noted among the offspring of sables; the least among black-and-tans and reds. We suspect also that a like gradation occurs in the amount and intensity of black and brown pigments in the black and the brown recessives of these series, though on account of the self color of these varieties this point is difficult of verification, except by breeding tests. From some tests which have been made and others which are under way, the evidence seems to show that blacks from sables and yellows have less intense young when crossed with agoutis, than do the blacks out of pure black-and-tan. Tables and curves for this cross will be given at a later time.

It is significant now that sables and black-and-tans may be synthesized by a cross of blacks out of the black-and-tan race with reds, showing that the black recessives carry the same differentiating element as do the black-and-tans. Such a cross produced 45 young, 20 of which were black-and-tan or sable, while 25 were black. The F_1 blacks were heterozygous for brown, *inter se* matings producing 32 blacks and 13 browns.

When a black which was heterozygous for brown was mated to a red, yellows falling in both the black and the brown series were produced as follows:

Black Series

Grade	1,	2,	3,	4,	5,	Blk.,	Total
Frequency	1,	1,	2,	0,	2,	1,	7

Brown Series

Grade	1,	2,	3,	4,	5,	Br.,	Total
Frequency	1,	2,	0,	3,	1,	6,	13

The element added in this last cross is plainly the brown gamete carried by the black. This brown gamete, however, has received something additional from the black-and-tan race, so that when red unites with this changed brown gamete the result is a darkening and intensification of the brown pigments to produce a brown-and-tan or brown-sable, a process quite parallel to that which produces black-and-tan and black-sable in the pure black × red cross.

A few crosses were made between pure-bred black-and-tan and brown, and although the numbers here are small, the indication is that the result will be the same as in the black × red cross. F_1 consisted of blacks and black-sables; the sables when back-crossed to browns gave approximately equal numbers of blacks (26) and browns (20) and also the two yellow series as follows:

Black sables (mean grade 3.5)	11	Brown sables (mean grade 4)	10
Yellows and sooties	3	Reds and yellows	10
Total	14	Total	20

This back-cross with the recessive brown gives a direct index of the yellow gametes of the F_1 sables. That they vary in darkness should be borne in mind during the discussion of the difference between black-and-tan and yellow.

The reds in suitable crosses showed the same tendency to produce fluctuating blends. Mated with creams they gave yellows of an intermediate shade (16) and recessive non-yellows (10). These light reds were bred *inter se* and tested by crossing with browns. The young (100 in number) fluctuated in intensity about the shade of the light red parent or parents. Full intensity was not recovered except in back-crosses. Hence red is likewise a form of yellow, differing from it by an added intensity which blends in crosses.

The foregoing evidence has merely pointed to the yellow nature of black-and-tan and red; has classified them and their derivatives among the yellows, and has hinted

at the possible difference between these forms and ordinary yellows. It is time now to inquire as to the real genetic nature of these mice, and to attempt a preliminary explanation of their differences from yellow. By far the largest number of mice have been bred and are being bred toward this end.

Let us consider first the black-and-tan variation. By its behavior it evidently forms two sorts of gametes, black-and-tan (yellow) and black. Each of these has an added something which makes the zygote into whose composition it enters darker than ordinary yellow or black. We may call this something "darkener"—be it singular or plural—and indicate the gametes by YD and BD. Red, similarly, forms gametes red (yellow) and brown; and these also show an addition which we may call "intensifier." The gametes of red are then YI and bI. The sables produced by red × black-and-tan can only be referable to a union of YD and bI, or YI and BD since YDYI is non-viable, and since YDbI and YIBD unions have been demonstrated in the brown × black-and-tan and red × black crosses, respectively, and have produced in both of these latter cases similar sables. The presence of the darkener and the intensifier in the same zygote weakens both and demonstrates their physiological and genetic independence.

The next point to be noted is that both darkener and intensifier are variable. All gametes formed by zygotes containing D or I are not equivalent in their D or I content. It is possible to demonstrate this for the darkener; the variable intensification from crosses with red cannot yet be as satisfactorily shown on account of the difficulties of grading. For light on the action of the darkener we may turn to the agouti crosses.

The ordinary wild house-mouse when bred pure, shows the agouti pattern and gray color with great uniformity. It possesses the black and yellow pigments of the black-and-tan mouse as well as brown pigment, but contains no factor to dilute or darken these pigments. These facts make it an ideal race with which to test for a suspected

darkener which acts on the black pigment of a yellow mouse.

Yellow, Cuénot showed, is an allelomorph of agouti and non-agouti. Black-and-tan, in turn, is not an alternative form of agouti like the light-bellied gray mouse, but a yellow, and hence should be allelomorphic to agouti. And such it is as far as its yellow component is concerned, but not as regards its darkener. The F_1 young from a cross of black-and-tan by wild agouti vary in darkness about a mode midway between black-and-tan and agouti. Black-and-tan we may regard as full darkness and assign to it an arbitrary grade of 6. Wild agouti we may regard as entire absence of this darkness and assign to it the grade 1. F_1 from a cross of these two has consisted of two sorts of young. (1) The first sort may be considered as the result of a union of the YD gamete from black-and-tan with the agouti gamete. These mice have been called sable agoutis; since they have the general pattern of sables. The bellies are yellow; the darkness of the dorsal hairs is variable through the same range as that of the sables, while all hairs on flanks, head and parts of the back are agouti ticked. These yellow F_1 young graded on the sable scale show the following distribution:

Grade	2,	3,	4,	5,	Total
Frequency	1,	15,	7,	4,	27

(2) The second sort of F_1 young may be called non-yellow and referred to a union of the BD gamete from black-and-tan with the agouti gamete. These are simply much-darkened agoutis. The belly is gray like the wild agouti and the flanks are agouti ticked. The head and middle of the back are covered by hairs which are black for most of their length, a very narrow yellow band being present near the tip, or in some cases lacking entirely in an area of hairs down the center of the back. This type is known as dark agouti and has also been graded according to darkness on a scale parallel but not exactly equiva-

lent with the sable scale. Grade 1 was taken as ordinary wild agouti; grade 6 was taken as a gray-bellied black in which the agouti pattern had been lost and in which the darkness was equivalent roughly to that of the black-and-tan. In grade 2 the extent of black in each hair is increased, and the wide yellow band diminished; in grade 3 the yellow is left only in a narrow band; in grade 4 the yellow ticking is lost from hairs in a streak down the center of the back and in grade 5 the area of all black hairs is extended to cover the whole back, ticking being limited to the sides of the body. On the basis of such a scale the F_1 dark agoutis were distributed as follows:

Grade	2,	3,	4,	Total
Frequency	8,	23,	5,	36

These F_1 dark agoutis bred *inter se* have produced 155 young, of which 109 have been dark agouti and 46 black, indicating that the F_1 dark agoutis were heterozygous for black. The distribution of 58 of these F_2 dark agoutis is as follows:

Grade	1,	2,	3,	4,	5,	Total	Mean Grade
Frequency	17,	21,	11,	7,	2,	58	2.2

By using as parents the darkest of these agoutis, regardless of generation, dark agoutis were obtained, which when three weeks old approximated the grade 6. They resemble all-black mice with gray bellies except for occasional ticked hairs on their flanks.

It will be remembered that all darkness in these dark agoutis was acquired originally from the BD gamete of a black-and-tan mouse, since the range of darkness in the wild agouti used has never been above grade 1. Careful grading of the young from matings among dark agoutis should then furnish information as to the variation in the "darkener." If the "darkener" is a multiple thing such matings should afford it opportunity to segregate or Mendelize. A tabulation of matings among all classes of dark

agoutis of the young born since the introduction of the grading scale follows:

DISTRIBUTION BY GRADE OF YOUNG PRODUCED BY DARK AGOUTI PARENTS OF VARIOUS GRADES

Parents	Grade Distribution of Young									
	1	2	3	4	5	5.5	6	Total Agouti	Mean Grade	Black Young
1 × 1	32							32	1.00	1
2 × 2	11	21						32	1.65	1
4 × 2	10	17	5	2				34	2.00	1
4 × 3			6	5				11	3.45	1
4 × 4			6	7				13	3.46	0
5 × 4		1	11	8	18	4	4	46	4.41	14
5 × 5		1	11	15	11	9	4	51	4.34	8
6 × 4		1	4	15	13	4	9	46	4.28	2
6 × 5			4	11	11	18	7	51	4.94	12
6 × 6				5	11	10	11	37	5.29	11
$F_1 \times F_1$ (3 × 3)[1]	17	21	11	7	2			58	2.20	22
6 × 1[2]	5	23	8	1	1			38	2.20	0

It can be seen from a glance at this table that the variation in amount of darkness is a continuous one, from a gray-bellied agouti dorsally all-black, through every possible gradation to a wild-type segregate. The continuous nature of the variation was noted throughout the grading of the dark agoutis in the ever-present tendency to create more classes for the young by adding half and even quarter grades, a temptation which was yielded to only in the grade 5.5, this grade being given to dark agoutis which showed many agouti hairs on shoulders and legs. There is nowhere any evidence of a simple unit-difference between wild agouti and the darkener derived from black-and-tan. The only segregation is that seen in grade 1 animals which bred true and gave no evidence of possessing the darkener.

The above statements are not intended to be final. To date the evidence indicates the presence of a continuously variable and non-Mendelian character which gives the appearance of a blend in cross-bred young, and which in the pure black-and-tan race has been added to yellows carry-

[1] From the cross black-and-tan × wild.

[2] Highest grade dark agouti × wild.

ing black and has darkened and increased their black pigment to the greatest possible extent. The evidence has failed to exclude wholly the possibility of interpreting the darkness of black-and-tan by multiple factors, but the continous nature of the variation in hybrid young would call for the postulation of such a large number of modifiers that this view could be neither proved nor disproved. The nature of the darkener is still to be determined, but its action on both agouti and non-agouti young seems to be to increase the total amount of black or brown pigment produced.

The experiments with the red race do not admit of as definite conclusions as were reached concerning black-and-tan, because of the difficulties of grading for intensity. It is safe to say, however, that red is a race in which yellow has been greatly intensified by a process similar to, though distinct from, that which has produced the darkness of the black-and-tan.

STATISTICAL WEIGHTING FOR AGE OF ADVANCED REGISTRY COWS

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Any study of milk production that is made from a statistical standpoint must necessarily be complicated, for the reason that advancing age in a cow up to the time she is mature enables her to produce more milk and butter fat. A further difficulty lies in the fact that after maturity the effect of age on production has not been determined with any degree of certainty. Whether or not the increase in capacity is directly in proportion to the advance in age; at what age is the maximum of production reached; what relation is there between age and per cent. of fat in milk, and at what age is a cow past the power of full productiveness, are all questions that need investigation in a broad way.

Necessarily, the various breed associations must have made some comprehensive investigations to enable them to fix standards for milk and fat production, and since the only extensive authenticated records that we have are records of these associations, this study was made for the purpose of determining if their records were consistent with their standards, and if these standards could be used as a basis for weighting cows of different ages.

Method of Collecting Data

Seven-day records only were used, these being secured from the Holstein-Friesian Blue Book, Vol. 24. For the purpose of future investigation all the animals in two direct lines of descent were tabulated, one from a female. the other from a male, both animals being noted ones in the breed. The names, herd book numbers, ages at time

of record, pounds of milk, pounds of fat, and per cent. of fat were all tabulated. Each animal was given an arbitrary number which denoted its position in the generation, and the position of all its direct ancestors in their respective generations back to the primary ancestor of the population. All advanced registry males were tabulated also and numbered.

Records Obtained

From the female, Aaggie Grace, No. 2618, H.H.B., only 456 advanced registry records were obtained in 10 generations. In order to secure these records about twice as many animals were tabulated, the others consisting of the A.R.O. sires and their daughters that had not themselves made A.R.O. records but had two or more A.R.O daughters.

The male, Paul De Kol, No. 14634, H.F.H.B., in 9 generations produced 9,639 female progeny with A.R.O. records. About twice this number of animals were tabulated to secure these records.

Tabulation of Data

Necessarily, before this large accumulation of data could be studied systematically, it was necessary to tabulate it in concise form, and for this purpose correlation tables were made for each population, each table involving a pair of variables. Thus age was compared to pounds of milk, age to pounds of fat, and age to percentage of fat; three tables to each population. From these tables the means of the characters in classes, class average deviations, population means, average and standard deviations and correlation coefficients were worked out. Then from these data, curves were drawn to illustrate its trend graphically.

Results

The correlation tables I and II, compiled for the purpose of studying the frequencies and distributions of the population originating in the male, Paul De Kol, are not

shown here. The one and one and one half year class and the classes over ten years of age were small. For this reason unbalanced and irregular results would be expected for these classes, and by referring to the curves it will be seen that the premise was justified. The two and three year classes were represented by 1,690 and 1,346 individuals, respectively.

Table III gives the average deviations, mean pounds of milk, standard deviations, correlation coefficients and regression coefficients of the population with respect to age and pounds of milk and pounds of fat. Although the mean age is four years, the three and one half year class actually reached the mean pounds of milk of the population, as can be seen from Table IV. Correlation probably amounting to causation is shown in the tables up to six years of age, and after that age is reached the correlation is practically zero.

TABLE III

	Correlation of Pounds of Milk to Age	Correlation of Pounds of Fat to Age
Average deviation	69.8	2.91
Standard deviation	92.4 ± 0.449	3.65 ± 0.018
Mean pounds	395.5 ± 0.635	14.00 ± 0.025
Mean age	4.0 ± 0.013	4.0 ± 0.009
Correlation coefficient	0.604 ± 0.004	0.57 ± 0.005
Regression weight to age	29.84 ± 0.0006	1.11 ± 0.00003
Regression age to weight	0.012	0.29
Coefficient of variability C	23.4 ± 0.001	26.0 ± 0.001

Table IV. This table gives the means, average deviations, and plus deviations of the different age classes for both milk and fat production. From these tables the curves for milk and fat production were plotted. They formed also the basis for calculating the curve which is used as a comparison with the Holstein-Friesian curve of fat and milk requirement. These tables also afford an interesting study from the standpoint of capacity of cows for milk production at different ages.

Considering first the curves for milk production (Fig. 1) it will be noted that curve 1, which represents the

pounds of milk required by the Holstein-Friesian Association, must be calculated from the pounds of fat required. This was done by taking the average per cent. of the whole population and calculating the number of

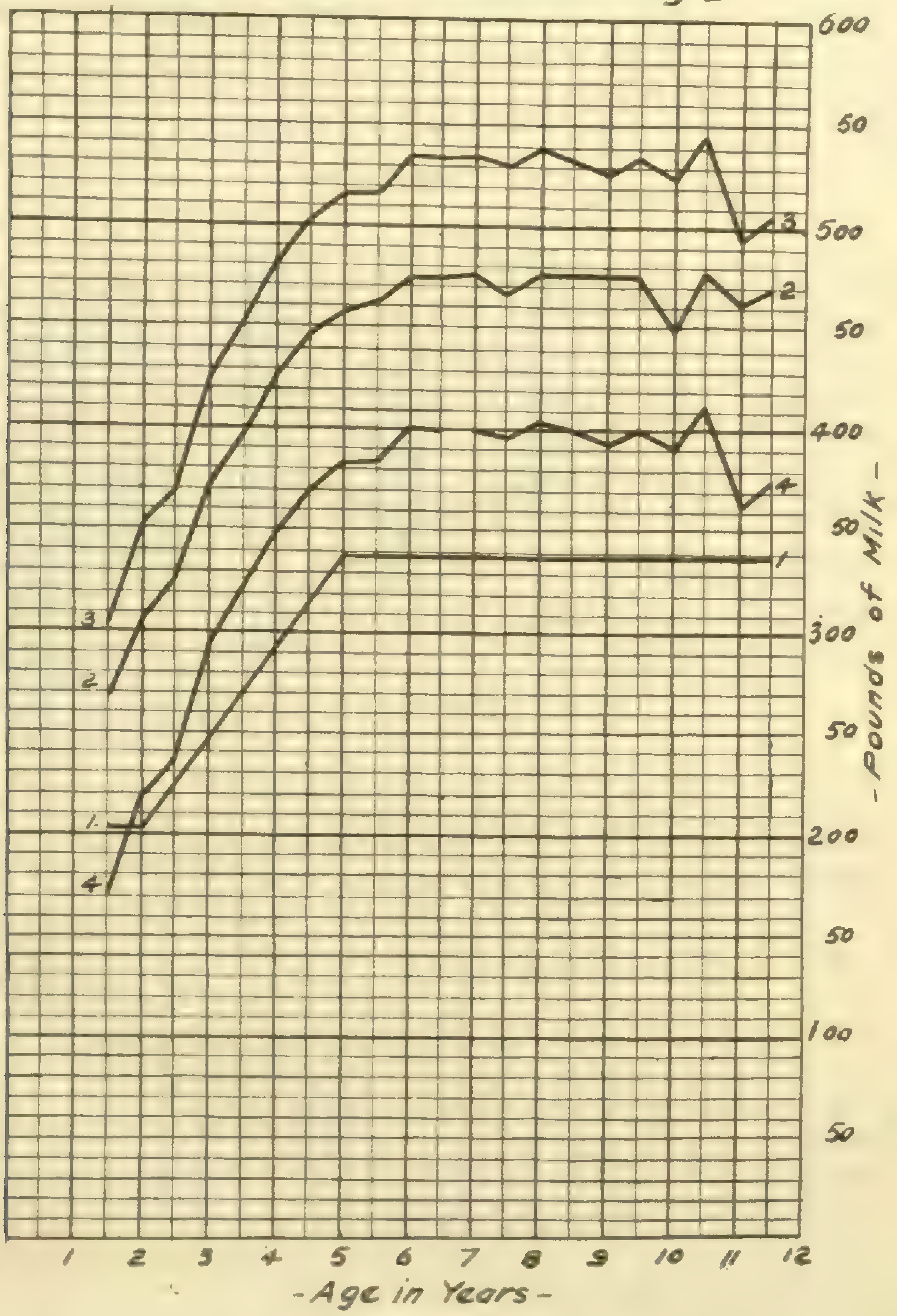
TABLE IV

Age, Years	Milk Production				Fat Production			
	Means	Av. Dev.	+ Dev.	Curve 4	Means	Av. Dev.	+ Dev.	Curve 4
1½	268	34	302	170	8.9	1.3	10.2	4.76
2	308	44	352	220	10.7	1.74	12.44	7.00
2½	326	43	369	237	11.3	1.76	13.06	7.62
3	372	56	428	296	13.0	2.14	15.14	9.70
3½	396	55	451	319	12.8	2.37	15.17	9.73
4	428	54	482	350	15.2	2.48	17.68	12.24
4½	446	57	503	371	15.5	2.3	17.80	12.36
5	458	59	517	385	16.4	2.36	18.76	13.30
5½	461	56	517	385	16.4	2.3	18.7	13.24
6	474	62	536	404	17.0	2.55	19.55	14.09
6½	474	60	534	402	16.8	2.43	19.23	13.77
7	476	60	536	404	16.7	2.3	19.00	13.54
7½	466	64	530	398	16.8	2.69	19.49	14.03
8	475	65	540	408	17.0	2.54	19.54	14.08
8½	476	58	534	402	16.6	2.46	19.06	13.60
9	476	51	527	395	17.1	2.43	19.53	14.07
9½	475	60	535	403	17.0	2.48	19.48	14.03
10	449	76	525	393	16.6	2.7	19.30	13.85
10½	477	68	545	412	16.7	3.1	19.80	14.33
11	461	35	496	364	16.0	2.0	18.00	12.53
11½	470	36	506	374	15.7	1.43	17.13	11.66
12								

pounds of milk, having the average per cent. that would be necessary to make the required number of pounds of fat. The reason for using the average per cent. of fat of the whole population as a basis for calculating the Holstein-Friesian Association requirement curve was that since the correlation coefficient between age and per cent. of fat was so small in a table shown subsequently for another population, and since the popular concept is that per cent. of fat is not influenced by age, we felt justified in using it. Attention is called to Table V, which does not bear out this assumption entirely. For milk and fat requirement, however, there is a strong correlation to age, so the classes were considered separately, each class having its own mean and deviation. Curves 2, 3, and 4 were based on these class means and deviations. Curve

No. 2 is the mean of the population. Curve No. 3 is the plus deviation from the mean. *Curve No. 4 is a curve*

Curves of Milk Production —Fig. I



which was plotted to show what the requirements ought to be if the means, deviations and varying capacity of the different classes are taken into account. In plotting this curve it was necessary to consider the basis upon which the minimum requirements of this population ought to be placed.

The minus deviation point can not show what ought to be required of the class as a minimum, for such point would weight individuals inversely in proportion to their capacity. A greater deviation from the mean of the class

indicates here greater capacity for production of that class, and as the capacity for production of the class increases, so should the requirements increase. Therefore, the curve of minimum requirement should be represented as following the curve of plus deviation in character and should be in a minus direction from the mean.

In order to conform to these conditions some basis must be established for calculating the minus points of the curve, or, in other words, the minimum requirements for each class. The average deviation of the whole population seems to be the logical basis upon which the minimum requirement should be based, for by its use the whole curve may be lowered an amount corresponding to the average deviation of the whole population below the mean of the population. The average deviation from the mean of the whole population is 69.8 pounds of milk. If all classes are to be given the benefit of the average deviation the calculation should start from the point at which the means are at the maximum, which is about the six-year class. Hence the six-year class is allowed as the minimum requirement, the 69.8 pounds below the mean of the class and the requirements of the other classes are worked out from this point to conform, as said before, to the maximum deviation curve.

An inspection of these curves brings out the following points:

That the official requirements weight animals of an age from 18 to 21 months too heavily. The curve indicates that they are entitled to a reduction as great as for any other age. For the purpose of discouraging such early breeding, however, the requirements for this particular class should be prohibitive and they are.

That the production increases up to at least *six years* of age instead of five, which the Holstein-Friesian Association requirements set as the maximum age production.

That for this reason the 5- to 6-year-old animals and possibly the 7- to 8-year classes have an advantage over all other classes.

That a comparatively small number of animals made

the requirement after 9 years of age, hence by selection, only the best animals were retained, thus drawing the curve down almost to a straight line. The tendency of the curve, however, is to recede, showing that the animals of these ages should not be weighted as heavily as younger animals. A study of a number of representatives of the whole breed would be necessary to determine this point.

One of the most striking points shown by these data and one which substantiates the opinion of practical breeders of Holsteins, also brought out in the practical investigations of Eccles,[1] is the difference in production and capacity between 2- and 2½-year-old and 3-year-old cows. The difference in the means of the production between 2 and 2½ years was 18 pounds only, while between 2½ and 3 years it was 46 pounds, or a total of 64 pounds between the 2- and 3-year classes. Between the 3- and 4-year classes the difference is almost as great, being 56 pounds, but the deviation of the latter class is not quite as great as the former. This seems to indicate that the 3-year animal is still at a disadvantage by reason of its immaturity in growth and body development. That the average deviation of 2½-year class was 43 pounds while the 3-year class deviated 56 pounds is significant also and leads to the conclusion that at 2½ years of age the Holstein is still growing, and this, combined with the great strain of milk production, limits the capacity of the class.

It may be said by some that few 3- and 4-year-old animals are tested for advanced registry in comparison to two year olds and aged animals, and in consequence of this, only the best of the class make the requirements. This is not borne out by the data, the number in the 3-year-old class being second largest of all animals.

Curves of Fat Production

A study of the curves based upon the actual fat production of this population (Fig. 2) brings out a number

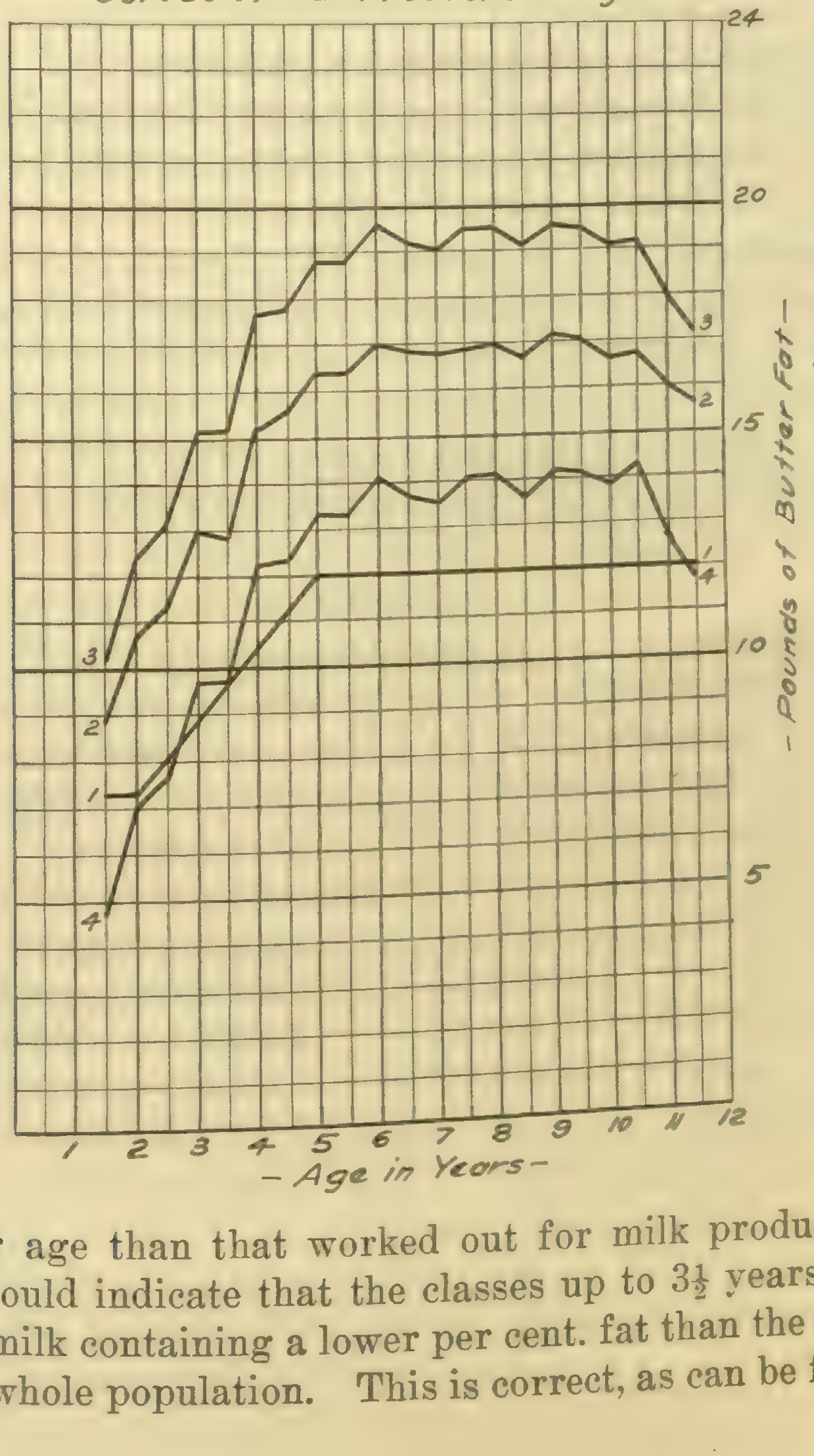
[1] Bul. No. 135, Missouri Agricultural Exp. Station.

of points, many of them corroborating those brought out in the discussion of the milk-production curves.

Owing to the variation of the weight classes in per cent. fat, the curves of milk production and fat production agree very well when compared with the Holstein-Friesian Association requirement curve.

The requirement curve in fat production (No. 4) crosses the Holstein-Friesian Association curve at a

Curves of Fat Production - Fig. II



greater age than that worked out for milk production. This would indicate that the classes up to 3½ years produced milk containing a lower per cent. fat than the mean of the whole population. This is correct, as can be found

from the means of the classes. (See average per cents. of class means, Table V.) A similar condition obtains with the age classes after ten years. It would appear from this that mature cows give milk slightly richer than immature cows, or than old cows past 10 years of age.

A rather peculiar condition with reference to the fat production curve is shown in the mean results of the half-year ages up to the 6½-year class. Each half-year class advances but slightly, if at all, from its preceding year class, then there is a sudden drop to the next full-year class. The milk production curves indicate the same condition, though to a lesser extent, and as previously noted, the frequencies in these half-year classes are not more than 60 per cent. of the full-year classes. No good explanation is offered for this. It might be inferred that a cow freshening at 2½ years is not much better able to withstand the strain of milk production than a 2-year-old, and that this condition continues. However, in many respects this theory does not appear sound.

Attention is called again to the points of curve 4 for fat production given in Table IV. This curve is plotted for the purpose of showing what the requirements ought to be according to the performance of cows that have made records. The animals involved in this curve represent 45 per cent. of all the A.R.O. records that had been made up to the time of publication of Vol. 24, hence the

TABLE V

AVERAGE PER CENTS. FAT OF THE CLASSES

Age, Years	Per Cent.	Age, Years	Per Cent.
1½	3.28	7	3.51
2	3.27	7½	3.61
2½	3.47	8	3.58
3	3.49	8½	3.49
3½	3.24	9	3.59
4	3.55	9½	3.58
4½	3.48	10	3.69
5	3.58	10½	3.50
5½	3.56	11	3.47
6	3.58	11½	3.34
6½	3.54		

numbers are ample. First, the means of the classes of this population were plotted. Then their ability to deviate in a plus direction, or, in other words, to produce more fat as individual classes was taken into account. The class that had the maximum production and deviation ability was allowed, as a basis for its minimum requirement, the full average deviation of the population in a minus direction from the mean, and finally the other classes that could not produce as much and had not the ability to deviate as much as this maximum class, were allowed the full minus deviation of the population plus the difference in deviation ability between their particular class and the maximum class which forms the apex of the curve.

If these fundamental allowances are fair, impartial and accurate, the curve is accurate, and the only question that remains is whether or not it should alter the requirements of the Holstein-Friesian Association. If curve 4 touches the Holstein-Friesian Association curve at any point and does not coincide with it throughout, then the latter should be changed. It *does* touch it at both beginning and end, showing that all classes after the 2½ years and up to 11½ years have an advantage over the others. This advantage is greatest for the classes between 5½ and 11 years of age.

The next consideration in connection with curve 4 is its application, and, when dealing with this, two things should be kept in mind; first, the practical, and secondly, the more concise and mathematical application. The practical application finds its expression in the endeavor of the Holstein-Friesian Association to make a uniform advance per day in the fat requirement for the seven-day test up to the age at which it was considered the maximum production was reached. Table VI compares the increase in the amount of fat required each year over that required in the previous year from two up to six years, with the increase in amount of fat that the year classes are *able* to produce as calculated from curve 4.

TABLE VI

Age, Years	H. F. A. Requirements		Curve 4 Requirements	
	Fat Increase, Yearly	Fat Increase, Daily	Fat Increase, Yearly	Fat Increase, Daily
2 to 3	1.6 lbs.	0.00438	2.70 lbs.	0.00740
3 to 4	1.6 "	0.00438	2.54 "	0.00696
4 to 5	1.6 "	0.00438	1.06 "	0.00290
5 to 6	0.0 "	0.0	0.79 "	0.00216

The table shows plainly that the daily increased requirement from 2 to 3 years should be 0.0074 instead of 0.00438, or 1.7 times as much. From 3 to 4 years should be 0.00696 instead of 0.00438, or 1½ times as much. From 4 to 5, 0.0029 instead of 0.00438, of nearly ½, and from 5 to 6 years, 0.00216 instead of no increase.

POPULATION No. 2

The second population tabulated is that which began with Aaggie Grace No. 2618, H.H.B., as the primary ancestress, and consists of only 456 animals. Correlation tables 7 and 8 are omitted, but 9 and 10 are given, and show all the data necessary for comparison with the previous population. Of course, it must be borne in mind that the comparison can not be too exacting, for this population is altogether too few in numbers to secure smooth results especially when comparing classes. In fact, the class means and deviations, Table IX, included only the classes up to 9 years because of the low frequencies after that age. If Tables III and IV are compared with 9 and 10, a remarkable agreement is noticed throughout, especially in the essential points which have been discussed.

The correlation table for age to per cent. of fat is not shown, but the coefficients of this table may be seen in Table X. The correlation coefficient is so small that it may seem negligible. but Table V shows that even with a low correlation, important points might be brought out if the data are sufficient.

No endeavor will be made in this paper to enlarge on the exact mathematical application of these data. This

will be taken up later in connection with a further study of the two populations.

TABLE IX

CLASS MEANS AND DEVIATIONS OF POPULATION 2

Age, Years	Age to Pounds Milk		Age to Pounds Fat	
	Mean Pounds Milk	Average Deviation	Mean Pounds Fat	Average Deviation
2	286	47.3	10.35	1.65
2½	310	48.6	11.35	1.63
3	392	52.5	13.16	1.87
3½	403	53.5	14.22	1.97
4	413	60.0	14.30	2.20
4½	470	57.8	16.5	2.28
5	469	56.6	16.42	2.30
5½	484	41.4	16.27	2.15
6	504	66.4	17.23	2.26
6½	406	93.9	17.75	3.37
7	480	59.2	16.45	1.56
7½	471	47.0	17.22	2.14
8	468	60.7	16.27	2.48
8½	500	40.0	18.20	3.92
9	447	47.6	15.00	2.29

TABLE X

POPULATION COEFFICIENTS; POPULATION 2

	Age to Pounds Milk	Age to Pounds Fat	Age to Per Cent. Fat
Means	405.4 ±2.8	13.89 ±0.113	3.455±0.014
Standard Deviations	90.39 ±2.02	3.593±0.08	0.436±0.009
Correlation Coefficients N	0.592±0.02	0.581±0.02	0.08 ±0.031
Coefficient of Variability C	0.223±0.005	0.258±0.006	0.126±0.003
Regression Weights to Age	26.51 ±0.042	1.057±0.002	
Regression Age to Weight	0.022	0.546	

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SHORTER ARTICLES AND DISCUSSION

ŒNOTHERA NEO-LAMARCKIANA, HYBRID OF *O. FRANCISCANA* BARTLETT × *O. BIENNIS* LINNÆUS

Œnothera neo-Lamarckiana is a name which I propose for a synthetic hybrid that so closely resembles *O. Lamarckiana* De Vries that I do not believe systematic botanists could separate it from the latter by characters which would enter into a specific description. This does not mean that the hybrid is the exact counterpart of any particular line of *Lamarckiana* carried forward by the geneticists who are working with this form for it must be remembered that there are numerous biotypes of this species differing from one another in matters of greater or less detail, and that workers with œnotheras know that *Œnothera Lamarckiana* of systematic literature is a collective or polymorphic species, various forms of which can be isolated as biotypes in the experimental garden. In my studies of the *Lamarckiana*-like hybrids I am selecting towards the type known to us through the work of De Vries and through the seeds distributed by him.

The parents of my hybrids are *O. biennis* from the sand dunes of Holland and *O. franciscana* from California. I have given in a recent paper[1] the contrasting characters of these species together with descriptions of hybrids in the first and second generations. These parents were chosen after several years of search among the œnotheras for wild species that might be crossed with the hope of obtaining *Lamarckiana*-like types. In this connection my attention was first called to *franciscana* by Prof. Bartlett. In *biennis* and *franciscana* together are suggested all of the essential taxonomic characters of *Lamarckiana* and there seemed good reason to expect that among hybrids of the second and later generations would be found forms with combinations of characters approaching very closely to the peculiarities of *Lamarckiana*. In this respect my cultures now in the fourth generation have yielded results quite as satisfactory as I have hoped.

[1] Davis, B. M., "Hybrids of *Œnothera biennis* and *Œnothera franciscana* in the First and Second Generations," *Genetics*, I, 197–251, 1916.

The results will, I think, show that *Lamarckiana*-like forms of *Œnothera* may be synthesized by simple crosses between wild species provided the parent species are selected with care. I believe that as the isolation of *Œnothera* types proceeds a number of different crosses will be found to give similar results, but this is the first successful combination that I have been able to study experimentally. My earlier work[2] with *Œnothera grandiflora* Solander and certain American wild types was planned at a time when *grandiflora* on historical grounds seemed to be a more important type in relation to the problem of the origin of *Œnothera Lamarckiana* than it does at present. That work was not so successful as the later in producing *Lamarckiana*-like hybrids for the reason that the parent species did not have as favorable characters for the end in view.

In spite of the recent paper of De Vries,[3] to which I have replied[4] in brief, my conviction is unshaken that Lamarck's plant, grown in the botanical gardens of Paris about 1796, was a form of *Œnothera grandiflora* Solander and can not be identified with the *Lamarckiana* of De Vries's cultures. On this view *Œnothera Lamarckiana* Seringe must pass into the synonymy of *Œnothera grandiflora* Solander. Neither am I convinced that other specimens in the collections of the Muséum d'Histoire Naturelle in Paris, particularly sheets of André Michaux and Abbé Pourret, may be referred to *Œnothera Lamarckiana*. I believe that the plant with which we are concerned in the experimental garden had a later origin and must bear the name of De Vries as its sponsor. At present our first certain date of the progenitors of *Œnothera Lamarckiana* De Vries appears to be about 1860, when the seed firm of Carter and Company in London introduced the plant to the trade.

Both De Vries and Gates have accepted my suggestion that Carter and Company obtained their material of *Lamarckiana* from some English station and not from Texas, as they state. We have no evidence that *Lamarckiana* ever grew in Texas and to me there is no evidence that it was ever native to America.

[2] Davis, B. M., "Some Hybrids of *Œnothera biennis* and *O. grandiflora* that Resemble *O. Lamarckiana*," Amer. Nat., XLV, 193–233, 1911. "Further Hybrids of *Œnothera biennis* and *O. grandiflora* that Resemble *O. Lamarckiana*," *Ibid.*, XLVI, 377–427, 1912.

[3] De Vries, Hugo, "The Probable Origin of *Œnothera Lamarckiana* Ser.," *Bot. Gaz.*, LVII, 345–361, 1914.

[4] Davis, B. M., "Professor De Vries on the Probable Origin of *Œnothera Lamarckiana*," Amer. Nat., XLIX, 59–64, 1915.

On the other hand, various races of *Lamarckiana* are at present growing wild in a number of English localities, the best known stations being on sand hills of Lancashire near Liverpool. A conspicuous *Œnothera* flora was present in this region as early as the beginning of the nineteenth century, as shown by an account in Smith's "English Botany," 1806. There seems to be no reason why *Œnothera Lamarckiana* might not have arisen in such a locality as a hybrid of species introduced into England possibly through Liverpool as a port of entry. Thus we are dealing with dates of introduction or origin that are reasonably close to present times; attempts to associate *Lamarckiana* with very early introductions into Europe appear no longer to have important support.

It is necessary to bear in mind this historical setting, since it may seem to my readers very improbable that *Œnothera Lamarckiana* should have arisen as a hybrid between *franciscana,* a species of western America, and *biennis* of Holland, England and other European countries. There is, however, nothing improbable in the possible meeting at Liverpool, with its world-wide commerce, of species of *Œnothera* from far corners of the earth. Furthermore, I should be the last to suggest that the particular races or species which give my *neo-Lamarckiana* have been the actual parents of the strains of *Lamarckiana* cultivated by De Vries. To strike the identical parental lines of such an assumed hybrid would in the case of the œnotheras be a most extraordinary piece of luck. It is remarkable that my results have proved so satisfactory; I have no doubt that other species crosses may sometime be made which will give hybrids as close or even closer to *Lamarckiana.*

The line of *neo-Lamarckiana,* which I now have in the F_4 generation from the original cross, was derived from a single selfed plant in the F_2 (14.53*c*), which fell well within the range of variation given by De Vries for *Œnothera Lamarckiana.* A description will later be published of this plant together with an account of its progeny through successive generations when these have been carried along somewhat further. The F_3 generation gave very few *neo-Lamarckiana* types, but these were closer to the large-flowered forms of De Vries's cultures. This F_3 generation was grown from earth-sown seeds and incomplete germination may have been responsible for the small proportions of *neo-Lamarckiana,* 7 in a total of 291 plants. The F_4 generation

was grown this summer from what seemed to be the most promising plant of the F_3 (15.53*a*). This culture was from seed germinated in Petri dishes and was complete, since the residue of ungerminated seeds were empty of contents. From 764 seed-like structures 668 seedlings appeared, but there was at once a large mortality among weaklings most of which were unable to free their cotyledons from the seed coats. Only 558 seedlings lived to be potted and a further mortality reduced the number that was set out in the garden to 549. Of these plants 198 as rosettes presented characters of *Lamarckiana* while 351 developed rosettes for the most part with narrower leaves suggestive of *franciscana.* All of the shoots from the 198 *Lamarckiana*-like rosettes have shown *Lamarckiana* characters of foliage, inflorescence, and flowers but about one fourth of the plants seem likely to persist this summer as rosettes. The group of *neo-Lamarckiana* in the F_4 generation is therefore large constituting about 36 per cent. of the total number of plants in the culture.

In the group of *neo-Lamarckiana* there is some variation, but the best plants are so close to the *Lamarckiana* of De Vries that I can only distinguish them by small plus or minus expressions of a few characters. Thus the central shoot is not so strongly developed proportionally to the side branches. The leaves are a little broader. Sepal tips do not spread so widely. Buds may not be quite so stout. The pubescence is somewhat heavier over certain portions of the plants. Time will tell whether even these small differences can be eliminated by judicious selection through succeeding generations.

It is of course not enough for critical bearing on De Vries's interpretation of the behavior of *Lamarckiana* that a hybrid should be synthesized taxonomically similar to it. Such a hybrid must also show a behavior parallel to *Lamarckiana* in its essential features. The two striking peculiarities in the breeding habits of *Lamarckiana* are (1) its ability to produce two types (twin hybrids) in the F_1 when mated to certain other species, and (2) its peculiarity of throwing through successive generations the same types of "mutants" in small, fairly constant proportions. Late in the season of 1915 reciprocal crosses were made between *neo-Lamarckiana* (15.53*a*) and plants of *biennis* and *biennis* (Chicago), forms which De Vries has used in his studies on twin hybrids from *Lamarckiana.* The conditions were not favorable for the technique of crossing and I am repeating the experi-

ments this year. However, I obtained from the cross *biennis* × *neo-Lamarckiana* two distinct classes of plants, (1) a narrow-leaved, smaller-flowered type with heavy pubescence and red papillæ (109 plants), and (2) broad-leaved forms, some larger-flowered, with a much lighter pubescence and few or no red papillæ (11 plants). Also, the cross *neo-Lamarckiana* × *biennis* (Chicago) gave two clearly defined classes distinguished at a glance by their size and foliage, (1) tall and narrow-leaved (64 plants), and (2) shorter and broad-leaved (11 plants). These crosses appear to have given twin hybrids and it should be said that the two groups were recognized and separated when the plants were in the rosette stage and that they consistently presented differences throughout all stages of their development. I shall from time to time make further studies of this behavior with different generations of *neo-Lamarckiana*. If *biennis* and *biennis* (Chicago) are pure species (a matter not yet established) this behavior would indicate that *neo-Lamarckiana* develops at least two classes of fertile gametes for both pollen and ovules. It thus seems probable that the behavior of *neo-Lamarckiana* when crossed to other species of *Œnothera* will parallel that of De Vries's *Lamarckiana* and thus support the view of several critics of the mutation theory that *Lamarckiana*, because it gives twin progeny in the F_1 of certain species crosses, must be itself a hybrid, producing different classes of gametes.

With respect to the ability of *neo-Lamarckiana* to throw "mutants" a most interesting situation is presented by its behavior this summer in the fourth generation. We have noted that a sowing of 764 seed-like structures gave 668 seedlings of which 198 developed as rosettes or mature plants into *neo-Lamarckiana*. Of the remaining 470 seedlings (668–198) only 351 lived to produce rosettes, a much larger group, however, than that containing the parent type, *neo-Lamarckiana*. We have then in the fourth generation *neo-Lamarckiana*, an impure or hybrid species, reproducing itself from at least 26 per cent. of its seeds. The exact percentage can not be told, for we do not know whether any plants of *neo-Lamarckiana* were among the 119 seedlings that died. In throwing a large progeny of a type very different from the parent F_3 plant, *neo-Lamarckiana* in the F_4 exhibited a behavior with strong resemblance to what Bartlett has described as "mass mutation." The types included a number of dwarf forms, but most of the plants resembled *franciscana*,

although generally stronger, larger-leaved, and with considerable variation in flower size. It should be noted that no forms similar to the parent *biennis* were present; this type of segregate seemingly is either not produced or appears but rarely.

The conditions of sterility in *neo-Lamarckiana* are likely to bear directly on the peculiarities of its behavior in comparison with that of De Vries's plant. My hybrids agree with *Lamarckiana* in having pollen about one half sterile, but the F_3 parent plant of this year's cultures showed seeds 87 per cent. fertile while the seed fertility of *Lamarckiana* is much lower, being reported by De Vries in extensive experiments as from 34.5–46 per cent. and for two lines of mine running in tests 26–30 and 32–36 per cent., respectively. The variation noted by De Vries is believed by him to depend upon whether or not the plants are heavily manured. The question at once arises may not the mass variation of *neo-Lamarckiana* in the F_4 be correlated with its very much higher seed fertility? What would happen if *neo-Lamarckiana* should develop a greater degree of seed sterility or if some lines should be segregated with seed sterility approaching that of De Vries's *Lamarckiana?* Would the plants eliminated come from among the *neo-Lamarckianas* or would they come from the assemblage of variants from this parent type? Should they come from the variants, as seems to me probable since *neo-Lamarckiana* is a sturdy plant, then a condition might be reached where the variants would appear rarely or in small proportions and this would parallel exactly the present behavior of De Vries's *Lamarckiana* in throwing its "mutants." I shall watch intently for indications in my cultures of increased seed sterility and among my plants of *neo-Lamarckiana* select steadily towards the higher degree exhibited by *Lamarckiana.* It is interesting that the last stages in the experimental synthesis of a *Lamarckiana*-like hybrid should be concerned chiefly with selection towards a definite degree of seed sterility.

Œnothera neo-Lamarckiana illustrates clearly my concept of an impure species of *Œnothera.*[5] It is a plant that breeds true in a proportion of its offspring but is heterozygous since it develops varied types of gametes as proved by the assemblage of offspring which differ sharply from the parent plant, and further indicated by its behavior in producing twin hybrids. The facts of a high degree of pollen sterility (about 50 per cent.) together

[5] Davis, B. M., "The Test of a Pure Species of *Œnothera,*" *Proc. Amer. Phil. Soc.,* LIV, 226–245, 1915.

with a certain amount of seed sterility indicate the probability that other classes of gametes are eliminated or fail to function and that possibly certain types of zygotes may be formed which are unable to live. *Œnothera neo-Lamarckiana* therefore shows itself to be impure or heterozygous because it develops different types of gametes even though the plant when selfed reproduces itself in a fairly large proportion of its progeny. This behavior seems to me quite the same in principle as that of De Vries's *Lamarckiana,* the only difference being that the total number of individual variants thrown by *Lamarckiana* is much smaller than those *at present* thrown by *neo-Lamarckiana.* However, as has been noted, the seed sterility of *Lamarckiana* is very much higher than that of *neo-Lamarckiana* in the F_4 generation, and it is my working hypothesis that this fact is at least partly responsible for the smaller numbers of variants produced by the former.

Œnothera Lamarckiana of De Vries's cultures seems to me best interpreted as an impure species producing regularly because of its heterozygous or hybrid nature a number of classes of gametes relatively few of which, because of the extensive sterility, both gametic and zygotic, are able to form viable seeds different from those that reproduce the species. *Œnothera Lamarckiana* breeds true in its high degree because only the gametic combinations that reproduce *Lamarckiana* survive the mortality visited on most of the gametes and zygotes. By this view *Lamarckiana* is very much the reverse of a representative pure species which De Vries has assumed it to be and its "mutating habit" is the result of its hybrid origin and heterozygous nature rather than a spontaneous expression of homozygous germ plasm. The fact that the same types of "mutants" from *Lamarckiana* are produced by successive generations in fairly stable proportions indicates that their differentiation lies in the mechanism of segregation in heterozygous germ plasm rather than in a sporting tendency (mutation) which would be expected to express itself in ever-varying ways and degrees.

It is worth noting how different is the conception, here expressed, of the constitution of a pure species from the view formerly and probably now very generally held. Formerly a species was considered pure if it bred true. Now we believe that a species may be impure and still breed very largely or even wholly true if a degree of sterility is present sufficient to render

abortive or infertile all types of gametes or zygotes that may be produced except the ones which carry forward the heterozygous line. The test of a pure species is then not that it should breed true (that is a corollary), but that it should produce gametes uniform except as they may differ with respect to the factors for sex characters.

In laying such great stress on the phenomena of gametic and zygotic sterility so very extensively present in the genus *Œnothera* it must not be supposed that we have as yet established the degrees to which sterility may be genetic in its character or to what extent it may be of a physiological nature. Only the sterility that has as its cause the failure of the reduction divisions to produce fertile gametes or the failure of the gametes to conjugate freely can properly be of a genetic nature. There is probably also a type of sterility due to physiological causes, as perhaps malnutrition, and this might affect gametes and zygotes which under favorable conditions would be fertile. We are very far from an understanding of the causes of sterility in *Œnothera*, to what extent cytological or to what degree physiological, and it would at present be most unsafe to carry lines of speculation very far in this field as regards the material under consideration.

Professor De Vries has expressed strongly a belief in the futility of my attempts to synthesize a *Lamarckiana*-like hybrid, taking the stand that unless the parent stock is known to be stable mutability might be inherited from one or both of the parent species, or that variants, the result of a cross from impure stock, might be mistaken for mutations. As a matter of fact *Œnothera biennis* is known to be unstable, producing a small series of "mutants," while *O. franciscana* has not been tested for its purity. Apparently Professor De Vries and I are working from assumptions that are far apart. The inheritance of a mutating habit such as that claimed for *Lamarckiana* would mean to me the inheriting of a heterozygous germ plasm running back to some hybrid origin. To me phenomena such as is exhibited by *Lamarckiana* in throwing its "mutants" indicates in itself the probability of heterozygous germ plasm. If this behavior is to be presented as evidence of mutation the purity of *Lamarckiana* must be established beyond all reasonable doubt and this in my opinion has not been shown. The tests of cross breeding, when twin hybrids result, and the very high degrees of gametic and zygotic sterility strongly indicate genetic im-

purity. And back of this is an obscure history for the material with no evidence it seems to me that *Lamarckiana* was ever present as a native species of any flora. The chief value which the study of my *Lamarckiana*-like hybrid may have for the problem of the origin and status of *Œnothera Lamarckiana* is likely to be a clearer understanding of how an obviously impure species, *neo-Lamarckiana*, may arise, a species which seems likely to present a breeding behavior parallel to that of *Lamarckiana*, and most important of all the significance of sterility in the working out of these results. It appears to me a matter of no vital importance to the status of a hybrid whether its parents are pure or impure. If markedly impure the problem of analysis for future generations merely becomes the greater. Since no species of *Œnothera* has as yet passed the tests for a pure species, we are at present in all of the *Œnothera* work talking of an abstraction when this concept is considered.

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August, 1916

STATISTICAL STUDIES OF THE NUMBER OF NIPPLES IN THE MAMMALS

IT is perhaps not unnatural that a subject of such fundamental interest as that of the nourishment of the young in the mammals should have attracted the attention of observers from the time of the Greek philosophers. It is only within the last few years that attempts have been made to solve various problems by the application of the statistical method to series of quantitatively recorded data.

The materials may be divided for convenience of review.

TYPE, VARIATION AND CORRELATION IN NUMBER OF MAMMÆ

The statement made by Parker and Bullard,[1] on the basis of their splendid series of data for swine, that the standard deviation of the number of nipples is 0.6906 in the males and 0.7905 in the females at once arouses the suspicion of a biometrician. The constants actually are:

[1] Parker, G. H., and C. Bullard, "On the Size of Litters and the Number of Nipples in Swine," *Proc. Amer. Acad. Arts and Sci.*, 49: 399–426, 1913.

	For Males	For Females
Mean	12.4365 ± .0182	11.9077 ± .0159
Standard Deviation	1.4800 ± .0128	1.2803 ± .0112
Coefficient of Variation	11.901 ± .105	10.752 ± .096

Thus instead of the females being "over 14 per cent. more variable than the males" they are in absolute terms actually .1997 ± .0175, or over 13 per cent., *less* variable. Relative variability as measured by the coefficient of variation is 1.149 ± .142 per cent. lower in the female that it is in the male. This lower variability of the female is also quite in evidence if the materials be split up into groups with regular and irregular arrangement of the nipples. Thus:

For "Regular" Class

Males	$\sigma = 1.485 \pm .017$,	C. V. = 12.03 ± .14
Females	$\sigma = 1.315 \pm .021$,	C. V. = 11.16 ± .13
Difference	0.170 ± .027,	0.87 ± .19

For "Irregular" Class

Males	$\sigma = 1.461 \pm .020$,	C. V. = 11.61 ± .16
Females	$\sigma = 1.210 \pm .016$,	C. V. = 10.02 ± .14
Difference	0.251 ± .026,	1.59 ± .21

However measured, the variability of the number of nipples in the female is always significantly less, *not greater*, than in the male.

Furthermore a rather noteworthy sexual differentiation seems so far to have escaped notice. The mean number of nipples for male pigs is in all cases higher than that for female pigs. Thus:

All Pigs

Males	12.4365 ± .0182
Females	11.9077 ± .0159
Difference	0.5288 ± .0242

Classified as Regular

Males	12.3425 ± .0233
Females	11.7849 ± .0214
Difference	0.5576 ± .0316

Classified as Irregular

Males	12.5833 ± .0234
Females	12.0777 ± .0232
Difference	0.5056 ± .0330

In all cases the males have on the average more nipples than the females. The regularity of the differentiation is brought out

by the accompanying table in which actual values have been reduced to *per mille* frequencies. Pigs with 12 nipples or fewer are preponderantly females; pigs with 13 nipples or more are preponderantly males.

Number of Nipples	Male	Female	Difference
8	.0	.3	+ .3
9	.6	1.7	+ 1.1
10	90.6	143.7	+ 53.1
11	162.7	217.9	+ 55.2
12	332.0	370.0	+ 38.0
13	167.6	154.5	— 13.1
14	163.4	82.8	— 80.6
15	49.3	20.0	— 29.3
16	29.8	7.1	— 22.7
17	3.0	1.7	— 1.3
18	1.0	.3	— .7
	1000.0	1000.0	

The correlation between the number of nipples on the two sides are:

Males	.6359 ± .0073
Females	.5419 ± .0088
Difference	.0940 ± .0114
All Pigs	.6063 ± .0055

The correlations are fairly high. Those for males seem to be slightly larger than those for females.

Correlation between the Number of the Young in the Litter and the Number of Mammæ in the Dam

The relationship between the number of young per litter and the number of mammæ in the female has at various times aroused considerable interest. As Pearl[2] has pointed out, two kinds of correlation are to be recognized. First, interracial correlation, that between the mean size of the litters and the mean number of mammæ in the females of a series of races or species. Second, intraracial correlation, that between the number of mammæ in an individual mother and the number of young that she bears.

It is the rather obvious interracial correlation that has given rise to such statements as that of Gegenbaur: "Die Zahl der Zitzen steht in inniger Beziehung zur Menge der Jungen." It

[2] Pearl, R., "On the Correlation between the Number of Mammæ of the Dam and Size of Litter in Mammals. I. Interracial Correlation," *Proc. Soc. Exp. Biol. Med.*, 11: 27–30, 1913.

was the problem of intraracial correlation with which Alexander Graham Bell[3] was dealing when he studied the fertility of the multi-nippled race of sheep at Beinn Bhreagh.

Notwithstanding the simplicity of the biological problem a certain amount of confusion seems to have arisen. Thus Parker and Bullard (*loc. cit.*) state:

> It is the chief object of our paper to discuss the relation of the size of litters to the number of nipples in the domesticated swine, *Sus scrofa Linn.*

But instead of determining the correlation between the number of teats of the sow and the number of her young they have actually calculated the relationship between the number of siblings in the litter in which a pig was born *and the number of nipples which she herself possesses!* Surely it should not require specialization in animal behavior to convince one that the teats which are of real service to a young pig are not its own, but those of its mother!

Pearl[4] has quite correctly determined the correlation between the number of nipples in the individual mothers and the number of young in their litters. This he finds to be very low,[5] $r = 0.195 \pm .086$.

It is rather difficult to agree with Pearl in his statement that

> It would seem, *a priori*, that natural selection should have operated to bring about a high correlation, both intra- and inter-racial between these two variables, size of litter and number of mammæ in the dam.

There seems no reason whatever to suppose that natural selection would tend to produce a correlation between the number of mammæ in the mother and the size of her litters *within a race, providing it has produced an average number of nipples suffi-*

[3] Bell, Alexander Graham, *Science*, N. S., 9: 637–639, *pl. 5*, 1899; *loc. cit.*, 19: 767–768, 1904; *loc. cit.*, 36: 378–384, 1912.

[4] Pearl, R., "On the Correlation between Number of Mammæ of the Dam and Size of Litter in Mammals. II. Intraracial Correlation in Swine," *Proc. Soc. Exp. Biol. Med.*, 11: 31–32, 1913.

[5] Wentworth (*Jour. Agr. Res.*, 5: 1148, 1916) records another very low coefficient on unpublished data, but does not state specifically whether it is between the number of mammæ of the mother and the number of her young as in Pearl's series, or between the number in a litter (weighted with their own number) and number of nipples in the individual pigs, as in the series of Parker and Bullard.

ciently large to maintain the race.[6] On the contrary, any theory of ontogeny or phylogeny which demands the existence of a mechanism to provide an embryo pig with the particular number of nipples which would agree closely with the number of young she may be destined to bear as an adult would seem to be not merely cumbersome, but unnecessarily teleological. Since male pigs have more mammæ than females, the cost to the organism is apparently not prohibitive! What one should expect as the result of the action of natural selection would, therefore, not be the development of a regulative mechanism to provide the mother with a number of nipples in close agreement with the size of her future brood, but the development of a number of nipples sufficiently large for the needs of the race.

Pearl's own data show only 7 out of 57 "disadvantageous" combinations, and the table as it stands takes no account of early deaths.[7] Furthermore, his series is small, only 57 individuals, and apparently hardly typical of swine as a class. Parker and Bullard on the basis of a thousand litters show that the (empirical) modal number of nipples is twice the modal number of young, and that the average number of nipples is much more nearly twice the number of young than in Pearl's short series. Thus the data of both Pearl and Parker and Bullard indicate in the words of the latter authors that "disadvantageous combinations in which the number of young pigs outrun the provision for

[6] Natural selection can not be expected to accomplish more for the development of any character than to bring it to and maintain it at a stage of development necessary for the survival of the species in competition with others. That correlation between the number of the young and the number of nipples is not necessary under conditions of domestication is shown by the classic observations of Minot on the guinea pig (*Jour. Phys.*, 12: 103, 1891) in which he pointed out that in his studies 143 litters showed a variation of from 1 to 8 in the number per litter, with a model frequency on 2 and an average of 2.5, although the number of developed mammæ is two.

That the number of young born may regularly exceed the number of nipples in a species persisting under natural conditions is shown by the recent studies of Hill and O'Donaghue on the marsupial *Dasyurus viverrinus* (*Quart. Jour. Micr. Sci.*, N. S., 59: 133–173, 1914) in which they have shown that a remarkable number of eggs are discharged from the ovary at each ovulation and that as a rule more young are borne than can possibly survive because of the limited accommodation of the pouch.

[7] Unfortunately trustworthy figures showing directly the mortality of new-born or recently born pigs seem not to be available. That such mortality is considerable is indicated by certain of the figures given for another purpose by Evvard.

milk, cannot be of frequent occurrence.'' The development of just such a ''factor of safety'' and not the origination of an intraracial correlation is, as emphasized above, just what one would expect of natural selection.

Natural selection, if operative, should, however, bring about an interracial correlation, and this is exactly what observant biologists have always noted and Pearl has expressed statistically by the value $r = .594 \pm .046$, with non-linear regression—a value distinctively higher than that for the intraracial relationship. Thus, as far as they go, these observations instead of evidencing against natural selection, actually show the very conditions to exist which might be expected as the result of the action of this factor of organic evolution.

Inheritance of Number and Arrangement of Nipples in Swine

Attempts at the Mendelian analysis of inheritance of number and arrangement of mammæ in swine have been made by Wentworth,[8] who has suggested that the presence of rudimentary nipples is a sex-limited,[9] sex-linked,[10] or sex-limited[11] character. His final stand is that the pair of rudimentaries posterior to the inguinal pair behave as a Mendelian unit character in heredity, but that somatically it develops in males, which are *RR* or *Rr*, but in the females only when they are *RR*, where *R* indicates the presence and *r* the absence of the factor for rudimentaries.

It is interesting to return to the sexual dimorphism with respect to number of mammæ demonstrated above on the basis of Parker's and Bullard's splendid series of data and to consider it in connection with the hypothesis advanced by Wentworth.

Pearson many years ago showed[12] that with continued random mating the distribution in any generation subsequent to an original random pairing of *RR* and *rr* individuals is

$$\tfrac{1}{4}RR + \tfrac{1}{2}Rr + \tfrac{1}{4}rr.$$

[8] Wentworth, E. N., ''Inheritance of Number of Mammæ in Swine,'' Rep. Am. Breed. Ass., 8, 1912.

[9] Wentworth, E. N., ''Another Sex-limited Character,'' *Science*, N. S., 35: 986, 1912.

[10] Wentworth, E. N., ''Sex-linked Factors in the Inheritance of Rudimentary Mammæ in Swine,'' *Proc. Iowa Acad. Sci.*, 21: 265–268, 1914.

[11] Wentworth, E. N., ''Rudimentary Mammæ in Swine a Sex-limited Character,'' *Science*, N. S., 43: 648, 1916.

[12] Pearson, K., *Phil. Trans. Roy. Soc. Lond., A*, 203: 59–60, 1904.

Both Pearl[13] and Jennings[14] have followed him in this point. If the thousand litters studied by Parker and Bullard come from a population homozygous and heterozygous with respect of a pair of rudimentary nipples in the 1:2:1 proportion and mating at random,[15] then three out of four males as compared with one out of four females should, if Wentworth's hypothesis be correct, show the pair of rudimentaries. Thus the average number of mammæ in the males should be 1 higher than in the females. As a matter of fact it is .529 ± .024 higher.

Further discussion on the basis of the present data would of course be idle.

In his largest paper Wentworth[16] has presented data which indicate sensible parental and grandparental correlations for number of mammæ. In view of the irregularity of the frequency distributions due to the modes on the even numbers and the smallness of the series, as well as the fact that the number of boars was very limited, little weight is to be given to the exact numerical values of his coefficients.

A more detailed analysis of the extensive series of data collected by Parker and Bullard may throw considerable light upon the problem of inheritance. The results must be expressed in terms of fraternal or sororal correlation. Those who are so obsessed with Mendelian theory that they are unwilling to learn anything about a series of data for which their method fails, should discontinue the reading of this review at this point.

Correlation between the number of nipples in siblings may be very readily found by means of intra-class correlation formulæ[17] involving first and second moments for the individual classes (litters).

Let x_m be the number of nipples in a male, x_f the number of nipples in a female pig, n_m the number of males and n_f the number of females in a litter of $n_m + n_f = n$ individuals. Let Σ denote summation within the litter and S a summation for litters. For any litter the moments are therefore $\Sigma(x_m)$, $\Sigma(x_m^2)$, $\Sigma(x_f)$,

[13] Pearl, R., AMER. NAT., 47: 606–609, 1913.

[14] Jennings, H. S., *Genetics*, 1: 64, 1916.

[15] Random mating of course applies only to the particular character in question, which is one which would hardly be consciously selected by any breeder.

[16] Wentworth, E. N., "Inheritance of Mammæ in Duroc Jersey Swine," AMER. NAT., 47: 257–278, 1913.

[17] Harris, J. Arthur, *Biometrika*, 9: 446–472, 1913.

$\Sigma(x_f^2)$. Since in a symmetrical intra-class correlation table the variates are weighted in an $(n-1)$-fold manner the fraternal correlation for males is given at once by direct summation from the data table of Parker and Bullard by the formula, written for simplicity in an entirely unreduced form,

$$r = \frac{\frac{S[\Sigma(x_m)]^2 - S\Sigma(x_m^2)}{S[n_m(n_m-1)]} - \left(\frac{S[(n_m-1)\Sigma(x_m)]}{S[n_m(n_m-1)]}\right)^2}{\frac{S[(n_m-1)\Sigma(x_m^2)]}{S[n_m(n_m-1)]} - \left(\frac{S[(n_m-1)\Sigma(x_m)]}{S[n_m(n_m-1)]}\right)^2},$$

or substituting actual values

$$r_{x_{m1}x_{m2}} = .323 \pm .019.$$

Apparently complex, the formula is really on closer inspection very simple indeed.

One altogether similar for the females gives the sororal correlation

$$r_{x_{f1}x_{f2}} = .373 \pm .018.$$

Thus the correlation for the females is $.050 \pm .026$ higher than that for the males.

For the cross correlations, that between number of nipples borne by male and female pigs of the same litter, the constant is given by

$$r = \frac{\frac{S[\Sigma(x_m)\Sigma(x_f)]}{S(n_m n_f)} - \frac{S[n_f\Sigma(x_m)]}{S(n_f n_m)} \times \frac{S[n_m\Sigma(x_f)]}{S(n_m n_f)}}{\sqrt{\frac{S[n_m\Sigma(x_f^2)]}{S(n_m n_f)} - \left(\frac{S[n_m\Sigma(x_f)]}{S(n_m n_f)}\right)^2}\sqrt{\frac{S[n_f\Sigma(x_m^2)]}{S(n_f n_m)} - \left(\frac{S[n_f\Sigma(x_f)]}{S(n_f n_m)}\right)^2}}$$

or

$$r_{x_m x_f} = .287 \pm .020,$$

a value apparently distinctly lower than that for either males or females alone.

If the correlation between the siblings be determined *irrespective of sex* the value is

$$r = \frac{\frac{S[\Sigma(x)]^2}{S[n(n-1)]} - \left(\frac{S[(n-1)\Sigma(x)]}{S[n(n-1)]}\right)^2}{\frac{S[(n-1)\Sigma(x^2)]}{S[n(n-1)]} - \left(\frac{S[(n-1)\Sigma(x)]}{S[n(n-1)]}\right)^2},$$

where the n and x without subscripts denote number of individuals per litter and number of nipples per individual, without reference to sex. Numerically

$$r_{x1x2} = .305 \pm .019.$$

This is lower than the relationship for either of the sexes individually considered, just as one might have predicted on *a priori* grounds from the low value of the cross correlation and from the differentiation in the number of mammæ in male and female pigs.[18]

The correlation coefficients here given show that there is a very material degree of resemblance with respect of nipple number in pigs from the same litter.[19] Indeed the correlation is about one third of the maximum value. Such correlation can be due only to differences in intra-uterine environment or to a strong inheritance of nipple number. The latter seems by far the more probable explanation.

J. Arthur Harris

18 Harris, J. Arthur, "On Spurious Values of Intra-class Correlation Coefficients Arising from Disorderly Differentiation within the Classes," *Biometrika*, 10: 412–416, 1914.

19 These values of the fraternal correlation will be but slightly influenced by the weighting of the individuals in the determination of the correlations, since nipple number is but slightly correlated with number in the litter.

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AMERICAN NATURALIST

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EXPERIMENTAL INTERSEXUALITY AND THE SEX-PROBLEM[1]

RICHARD GOLDSCHMIDT

KAISER WILHELM-INSTITUT FÜR BIOLOGIE, BERLIN

UNTIL a comparatively recent time the sex-problem was one of the white spots on the map of biology. There is hardly another problem which has been such a playground of dilettantism, and if we look through the older literature on the sex-problem we find almost as many philosophers and economists inventing sex-theories as there are biologists. But there is scarcely another problem in biology which in the brief period of a decade and a half has emerged from a state of absolute ignorance into one of most hopeful knowledge. The all-important step which has been made during this time is the complete insight into the elementary mechanism which pro-

[1] Evening lecture delivered at the Woods Hole Marine Biological Laboratory, July 28, 1916. The majority of the facts recorded here as well as the principle of their explanation have been published in a series of papers which appeared following a preliminary report in *Sitzungsber. Ges. Morphol. Physiol. München*, 1911, namely: Goldschmidt, R., ''Erblichkeitsstudien an Schmetterlingen,'' I, *Ztschr. induct. Abstammungsl.*, 7, 1912; Goldschmidt, R., u. Poppelbaum, H., ''Erblichkeitsstudien an Schmetterlingen,'' II, *Ibid.*, Vol. 12, 1914; Poppelbaum, H., ''Beitraege zur Kenntnis, etc.,'' *Ibid.*; Goldschmidt, R., ''A Preliminary Report, etc.,'' *Proc. Nat. Ac. Sc.*, I, 1915; Goldschmidt, R., ''Genetic Factors and Enzyme Reaction,'' *Science*, Vol. 43, 1916. See further the related chapters in Goldschmidt, R., ''Einführung in die Vererbungswissenschaft.,'' 2. ed., Leipzig, 1913, and in Correns, C., and Goldschmidt, R., ''Vererbung und Bestimmung des Geschlechts,'' Berlin, 1913. A complete account of the entire work, with the necessary illustrations, is in preparation. I am greatly indebted to Dr. R. A. Spaeth for revising this MS.

duces the normal distribution of the two sexes. To-day it is well known to every one how this insight was reached practically simultaneously by the study of Mendelian inheritance and the cytological investigation of the chromosomes and how the two solutions are in most wonderful harmony. The outstanding facts which I regard as one of the corner-stones of modern biology are familiar even to the beginner in biology. Cytologically one of the sexes, the heterogametic sex, contains only one X-chromosome, the other, the homogametic sex, two of them. Every one knows how the maturation division separates entire chromosomes and therefore the heterogametic sex produces two kinds of sex-cells, *i. e.*, with and without X-chromosomes, but the homogametic only one kind, all with X-chromosomes; and how chance-fertilization produces again the two parental combinations, that is, the two sexes. You are furthermore familiar with the fact that the Mendelian experiments yield practically the same result. If we substitute the term "sex-factor" for "X-chromosome" and "hetero-homozygous" for "hetero-homogametic," the facts are identical. One sex is heterozygous for the sex-factors, say Ff; the other homozygous, say FF; the first one produces two kinds of gametes, the second only one, and chance-fertilization results in equal numbers of the parental combinations. The results of experiments on sex-limited inheritance have shown, finally, that both sets of facts are the same thing, only expressed in different language, in other words, that the X-chromosomes are the vehicles for the distribution of the sex-factors. If we state further that there are animals in which the heterozygous sex is the female, and others where it is the male, we know the elementary facts from which any further study of the sex-problem has to start.

Now that we know the elementary mechanism of sex-distribution, can we regard the sex-problem as solved? I do not think so. What are the sex-factors and how do they determine sex? Are the two sexes clean-cut alternatives and is it therefore impossible to transform one

into the other, or are they nothing but limiting points of a series, which might approach each other or even become interchanged? These and many other questions can only be approached by experimental modifications of the normal sex conditions. There is one road towards this goal, the application of the well-known influence of the internal secretion of the sex-glands upon the sex-characters in castration- and transplantation-experiments, as well as the study of analogous experiments by nature. Another approach is rather an unexpected one; this has been followed in my experiments, an account of which I now have the pleasure of giving you.

Insect-breeders have long known that in crosses of species as well as of geographic varieties a comparatively high percentage of sexual abnormalities are produced. Furthermore, any collector of moths knows that similar abnormalities, usually called gynandromorphs or hermaphrodites, appear occasionally in nature and one of the moths in which these occur is the gipsy-moth. Since this moth has a very wide range of geographic distribution through very different climates a considerable difference of geographic varieties is to be expected. Furthermore, as is well known, the sexes are extremely dimorphic. Therefore, seven years ago I began hybridization experiments of European and Japanese gipsies. I was rather fortunate in striking the right forms which gave, from the outset, the most interesting results.

The first result was that crosses of Japanese females with European males yielded normal offspring, whereas in the offspring of the reciprocal cross, European female × Japanese male, all males were again normal, but all females showed in all parts of their bodies admixtures of male characters. I first called these animals gynandromorphs. But as this term is usually applied to animals with bilateral or antero-posterior or similar mosaic of the two sex-characters, it seems advisable to use another term for these forms, which in general repre-

sent a definite step between the two sexes.[2] The phenomenon shall therefore be called intersexuality. Further experiments now proved that intersexuality segregates, F_2 giving normal and intersexual animals. It was further shown that in some experiments the females remained normal and the males became intersexual. When the experiments were repeated, however, different results appeared. And as the material used came from different strains, suspicion arose that there are many different races of gipsies, differing in regard to those things which are responsible for the intersexuality. This suspicion was strengthened when it became probable that the peculiar thing responsible for intersexuality could be influenced by external conditions. If this is the case, conditions in Japan ought to be very favorable for an origin of such racial variation, as the Japanese islands show climatic conditions varying from an almost arctic to an almost tropical climate. These suppositions turned out to be correct, as was proved by the further study of these questions in Japan in 1914 and with the material brought from there to this country.

In order to make the results clearer and their bearing on the sex-problem more evident, I want to apply just the opposite method from that used in the research work, and to give first the general interpretation of the results, which differs only in a few points from that used since 1911, and then the experimental facts from which it has been derived. What we have to explain is that two nearly related forms, both normal in regard to sex-inheritance, produce, if crossed in one direction, normal offspring, in the other direction, normal males and intersexual females; (2) that, as we may now say, the degree of intersexuality is definite in a given cross, but different in different crosses; (3) that intersexuality shows Mendelian segregation; (4) that males may become intersexual too in certain crosses.

The explanation for these and the other facts later to

[2] The special meaning of this will be discussed in another paper in connection with very interesting new facts.

be reported is the following: Both sexes contain the anlagen for either sex. In both sexes, irrespective of the zygotic constitution, both anlagen might become patent. Which one is to appear depends entirely upon the quantitative relation of both.[3] If we apply the usual symbols and keep in mind that the female sex is here the heterozygous one we have the following formulæ:

$$\boxed{FF}\,Mm = ♀, \qquad \boxed{FF}\,MM = ♂.$$

(It will soon be explained why the female set FF is put in a square.) The female set as well as the male set act independently and with a definite quantitative strength. In order to have a convenient term we call this quantitative value of the sex-factors their potency or valency.

[3] The idea that sex-determination is a quantitative rather than a qualitative process is of course not new. Practically all writers about the cytology of sex-determination have developed such ideas, beside the well-known old and new metabolistic theories. Such a quantitative view was proposed by myself in 1904 ("Der Chromidialapparat, etc.," *Zool. Jahrb.* (An.), 21) and more fully developed in 1910 ("Kleine Beobachtungen, etc.," *Arch. Zellf.*, 6). Other such theories were proposed by R. Hertwig, 1905–07 ("Ueber das Problem der Sexuellen Differenzierung, etc.," *Verhdlg. deutsche Zool. Ges.*, 1905, 1906, 1907), based on his views about the nuclear-plasmic relation. Again others of a strictly quantitative character were discussed by E. B. Wilson ("Studies on Chromosomes," III, *Journal Exp. Zool.*, 3, 1906), by Th. Montgomery, Jr. ("Chromosomes in the Spermatogenesis, etc.," *Trans. Amer. Phil. Soc.*, 21, 1906), and by Th. Boveri ("Ueber Beziehungen des Chromatins zur Geschlechtsbestimmung Sitzber. physikal. medizin. Ges. Würzburg," 1908–09). A full discussion of the relative values of qualitative and quantitative views in regard to sex-determination is given by Th. H. Morgan ("A Biological and Cytological Study, etc.," *Jour. Exp. Zool.*, 7, 1909), who decides for the latter. However, I think that all these authors no longer cling to the details of their former views. The first attempt to prove a quantitative theory of sex-determination experimentally has been made by R. Hertwig in his well-known experiments with frogs (since 1905). Quite another type of theory, practically the same as is to be used in this paper, combining the quantitative view with the Mendelian and cytological results, has been developed on an experimental basis in my papers from 1911 and 1912 (*l. c.*) and has been adopted by many writers (*i. e.*, Doncaster and Harrison, Standfuss, Witschi). A third view has since been developed by Riddle in a series of preliminary papers (Carnegie Year Book, 1913, *Science*, Vol. 39, 1914, AMER. NATUR., Vol. 50, 1916, etc.). His theory is based partly on chemical studies of pigeon eggs, partly on hybridization experiments with doves. It is not impossible that in the latter something like intersexuality is produced, if I understand the short accounts thus far published.

If you like to form a definite idea you might assume that the potency means a certain concentration of enzyme which acts according to well-known laws.[4] In order to make the situation clear we assume that we are able to measure their potency. And we find that the female factorial set $\boxed{FF}$ is 80 units strong and every male factor $M = 60$ units. Then in the female formula $\boxed{FF}$ Mm the female set overpowers the one M present by 20 units, whereas in the male formula $\boxed{FF}$ MM the two Ms with the value of 120 are 40 units stronger than $\boxed{FF} = 80$. Now we face two possibilities. Either the slightest preponderance of one set over the other, say by only one unit, is sufficient to determine the male or female sex, or there is a definite minimum of preponderance necessary—we call it the epistatic minimum—beyond which one or the other sex appears. Let us now suppose this minimum to be 20 units. Then of course 40 units are left between the two extremes male-female preponderance. If we call the difference value between the male and female factorial set e, then we have a female, when $\boxed{FF} - M = > 20$ and a male, when $MM - \boxed{FF} > 20$, or in other words the limiting values for e for the two sexes are $+20$ and -20. We can now express this conception graphically in the following diagram, where the values

FIG. 1

of e are arranged on a straight line. Individuals to the right of $+20$ are females, to the left of -20 are males. But what of the intermediate points? These are the intersexual animals; if they are heterozygous for M they are intersexual females and if they are homozygous for M they are intersexual males. How does this diagram now

[4] For proof that this conception comes near the truth see Goldschmidt, R., "Genetic Factors and Enzyme Reaction," *Science*, N. S., 43, No. 1099, 1916. Very important new facts will be published later, which will probably enable us to replace the symbolistic Mendelian language, used here, by more definite physico-chemical conceptions.

explain the fundamental experiment? Suppose we have two races both normal in regard to the quantitative behavior of their sex-factors, but with different absolute values of the potencies. The values are supposed to be the following:

$$\begin{array}{lcc} & \text{Weak European Race} & \text{Strong Japanese Race} \\ \text{♀} & \boxed{FF}\ Mm & \boxed{FF}\ Mm \\ & 80 \quad 60 & 100 \quad 80 \\ \text{♂} & \boxed{FF}\ MM & \boxed{FF}\ MM \\ & 80 \quad 60\ 60 & 100 \quad 80\ 80 \end{array}$$

It is evident that both races, if bred true, behave normally. Here we must now add that the female factors or anlagen are inherited exclusively maternally, without any paternal influence. Therefore the square. But the M's are typical Mendelian sex-factors. Let us cross now a Japanese ♀ with an European ♂. F_1 is then

$$\begin{array}{lclc} F_1\ \text{♀} & \boxed{FF}\ Mm, & F_1\ \text{♂} & \boxed{FF}\ MM. \\ & 100 \quad 60 & & 100 \quad 80\ 60 \end{array}$$

The value e is then + and — 40, the offspring is normal. The reciprocal cross European female with Japanese male gives

$$\begin{array}{lclc} F_1\ \text{♀} & \boxed{FF}\ Mm, & F_1\ \text{♂} & \boxed{FF}\ MM. \\ & 80 \quad 80 & & 80 \quad 80\ 60 \end{array}$$

Now we see that in the female e or $FF - M = 0$. The animal is intersexual, exactly half-way between male and female.

Instead of deriving further theoretical expectations we shall now see how the experimental facts fit these general conceptions of a quantitative nature of sex-determination. The first point is of course the question of the different absolute and relative potencies. There are primarily two ways open for testing it. One is to influence these potencies experimentally. Only a few preliminary steps in this direction have thus far been made,

which may be omitted here. Another way would be to find many races which differ constantly in regard to the potency of these factors, which could be shown by the results of cross-breeding. The expectations are, then, that with increasing value of M, in crosses, where these races are used as males in combination with "weak" females, a corresponding type of female intersexuality must appear, giving a complete series from femaleness to maleness. And if we can find a race with so high a potency of M, that the combination lies beyond the epistatic minimum for maleness, then all the would-be females will be transformed into males. I now have at hand such races from Europe and Japan and can produce at will and in 100 per cent. of the offspring all grades between the two sexes. Thus we have one Japanese race, the race G, medium strong in regard to the potency of the factor M. If we cross these males with females of the Japanese race K, which shows comparatively low potency of the female factorial set, all would-be females in F_1 are slightly intersexual. We might put them at the point + 15 in our diagram (Fig. 1). Their antennæ are feathered, but less than in males, a portion of the wings assumes the brown color of the male, there are not as many eggs as in a normal female, but the mating instincts as well as the copulatory organs are still female and the eggs may be normally fertilized. Then there is a European race F and a Japanese race H, both with a still lower potency of the female factorial set. If we cross these with the same Japanese males G we get somewhat slighter female intersexuality. All secondary sex-characters are more male-like; the instincts are still female and the animals attract the males and mate. Then one of the characteristic hairy egg sponges is laid, but it contains no eggs, only hairs. The copulatory organs are already changed in the direction of the male and no successful mating and egg deposition is possible, although the abdomen is filled with ripe eggs. Then there is another European race F with very low potency of the female factors. If we mate these with the same males G,

intersexual females appear which are more than half-way between males and females. The secondary sex-characters are almost male. The instincts and behavior are about intermediate between the sexes. Males are scarcely attracted or not at all and no mating occurs. The copulatory organs show the strangest combinations of the male and female types, but there are still typical but rudimentary ovaries left. There is now another Japanese race in my possession, the race X. This one exhibits a still higher potency of the male factor M. If we cross this one with the European race F, a still higher degree of intersexuality appears. Now we have animals which externally are almost indistinguishable from true males. But certain characters, especially in the copulatory organs, still show their female origin. The instincts are entirely male and they try—always unsuccessfully—to mate with females. But the most interesting feature is the sex gland. This is a body looking externally like a testis, but showing in sections every single step between an ovary with nothing but immature eggs through a mixture of ovarial and testis tissue to a real testis. This is of course the highest grade of intersexuality which can be reached. The next step would be the complete transformation of the would-be females into males. And this can be obtained too. I have two Japanese races O and A which show such a high potency of M that crossed with any European females and the Japanese females H they produce nothing but males, all would-be females being converted into males.[5]

[5] It might be added here that two different lines of female intersexuality can be distinguished in regard to the most conspicuous feature, the color of the wings. In one line intersexuality begins with white wings, then dark cunei appear on the wings; they grow larger and larger, forming streaks along the veins until finally only fine white spots are left on a dark wing. The second series shows even in the first grades of intersexuality male color all over the wings without any streak formation. Which type appears depends on both races involved, and is due to physiological conditions in regard to pigment formation which are not yet entirely clear. The intersexual males always exhibit the first type. Color photographs of an almost complete series of the second type are given in my papers from 1912 and 1914. Photographs of the first type in the female series are not yet published. A complete series of the transformation of the female copulatory

I think these facts alone would be sufficient to prove that the above given quantitative conception of sex-determination is the right one. But we can quote furthermore a real experimentum crucis. We have seen that the very weak European race F crossed with the medium strong Japanese males G give a fairly high grade of intersexual females. In our diagram of the values of e we might put them at the point —10 (Fig. 2). The same

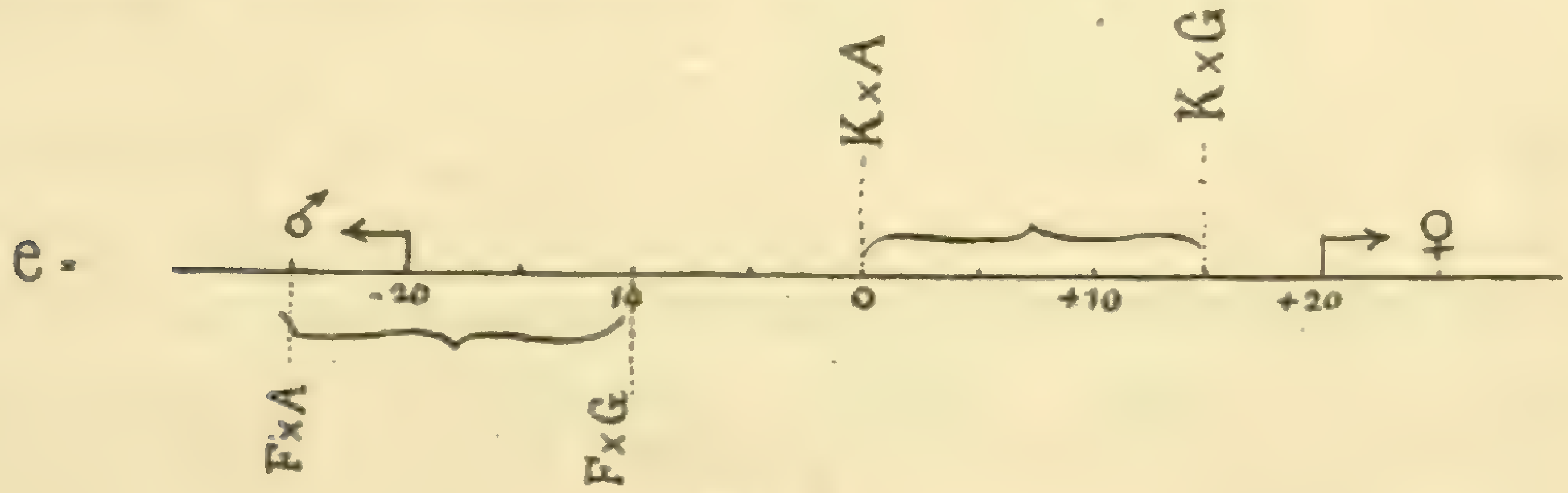

FIG. 2

weak females crossed with the very strongest males A gave nothing but males, the would-be females standing now on the point, say —25. Now the strongest of the weak races in regard to FF, *i. e.*, the Japanese race K, gave with these same males G slight female intersexualism, say at point +15. If our conception is right, the same females, crossed with the very strong males A, ought to give medium intersexual females, to be put at the point 0. And this is the actual result.[6]

Now we have reached a point where we want to know how the complexes [FF] and M are inherited. Anybody familiar with Mendelian analysis knows which results are to be expected if F and M are independent Mendelian factors. These tests, so far as fertility allows them to be

organs based on our material is pictured in the paper of Poppelbaum (*l. c.*). Photomicrographs of the transition from an ovary to a testis are given in my paper of 1914. In regard to the occasional appearance of a single normal female in cultures where all females are transformed into males, see our publication in the *Proc. Nat. Ac.* It was supposed that they originated from a case of "non-disjunction." Their behavior in breeding does not agree with this supposition but suggests another explanation, which at the same time explains the occasional appearance of intersexual males in nature. Details can not be given without a lengthy discussion.

[6] It might be added that a similar experiment can be performed with the Massachusetts race, which behaves about like the Japanese race K.

made, prove, of course according to expectation, that the factor M is Mendelian and carried in the X chromosome. All results of F_2 and back crosses agree in this point. But what is [FF]? Originally I believed that I could prove its Mendelian character too. This was a mistake, produced by the interference of another phenomenon, connected with the wing-color inheritance. The experiments made since my first communications prove thus far that the complex [FF] is inherited only through the mother, maternal grandmother, etc., *i. e.*, in the protoplasm of the egg.

We are now prepared to consider the production of the intersexual males, thus far omitted in our discussion. A series of them has been produced extending almost to femaleness. They are easily distinguishable from intersexual females (a point which is of special interest; one explanation being that factors contained in the Y chromosome are responsible for it). The intersexual males always exhibit the first of the above-mentioned (foot-note page 713) types in regard to wing coloration, white streaks appearing on the dark wings. While the wings assume more and more the female shape, the dark color becomes confined gradually to the wing venation, and in the extremest types thus far bred only a few brown spots appear upon some veins. All the other characters, like size of abdomen and copulatory organs, change hand in hand with this. But the behavior of the sex glands is not yet clear. In low grades of male intersexuality the testis always contains some ovarial tissue.[7] But the highest grade intersexual males of almost female exterior contained a paired[8] sex gland of somewhat testis character. filled with giant bundles of apyrene spermatozoa, and containing no eggs.

If we now deduce from our general interpretation how these interesting males might be produced, we realize that

[7] See picture in Poppelbaum's paper. The lower grades of male intersexuality are photographed in our papers from 1912 and 1914. Pictures of the higher grades are not yet published.

[8] The ripe testis is an unpaired organ.

they are expected to appear when $\boxed{FF}$ is comparatively high in potency and MM comparatively low. There are two possibilities for this event. (1) If we revert to our original example of a cross between weak European and strong Japanese races, we have in one direction:

$$\begin{array}{cccccc} \text{Eu.} \ ♀ & & \times & \text{Jap.} \ ♂ & & \\ \boxed{FF} & Mm & \times & \boxed{FF} & MM. & \\ 80 & 60 & & 100 & 80 & 80 \end{array}$$

As $\boxed{FF}$ is inherited maternally, any generation of this cross will have the weak set $\boxed{FF} = 80$. There is no combination of two M's possible which is not at least 20 units higher than FF, therefore no intersexual males can occur. Now take the reciprocal cross:

$$\begin{array}{cccccc} \text{Jap.} \ ♀ & & \times & \text{Eu.} \ ♂ & & \\ \boxed{FF} & Mm & \times & \boxed{FF} & MM. & \\ 100 & 80 & & 80 & 60 & 60 \end{array}$$

The F_1 males are $\underset{100}{\boxed{FF}}$ $\underset{80\ 60}{MM}$ and therefore normal, as $e = -40$. F_2 from this cross has again the maternal and grandmaternal set $\boxed{FF} = 100$. The Mendelian factors MM are recombined and we get the combinations:

$$\begin{array}{ccc} MM & \text{and} & MM. \\ 80\ 60 & & 60\ 60 \end{array}$$

The latter males are therefore:

$$\begin{array}{cc} \boxed{FF} & MM. \\ 100 & 60\ 60 \end{array}$$

This means that e has just the limiting value -20. It follows, that if we have two races, in which the relative values differ only slightly from this example to the disadvantage of the weaker M, say $M = 59$ instead of 60, male intersexuality is to be expected in the F_2 generation of a cross, where the mother belongs to the stronger race. This is indeed one of the actual facts.

According to the above derived formulæ these intersexual males ought to number exactly one half of the

male individuals. But this has so far never been the case. In order to understand it we have to point now to a fact which we omitted in our previous discussions. We have located the intersexual animals, males and females, at a certain point between the two sexes, as represented in the two diagrams. But in fact these points are only the mean of a certain range of variation in regard to the grade of their intersexuality. Whether this means that the potencies of the factors are variable or only their ultimate effect shows variation produced through influences during the development, is a question not to be discussed here. The fact is, however it might be caused, that the value of e as the measure of intersexuality, is variable around a mean. If this is the case, the following expectations are to occur (Fig. 3): If the values of e

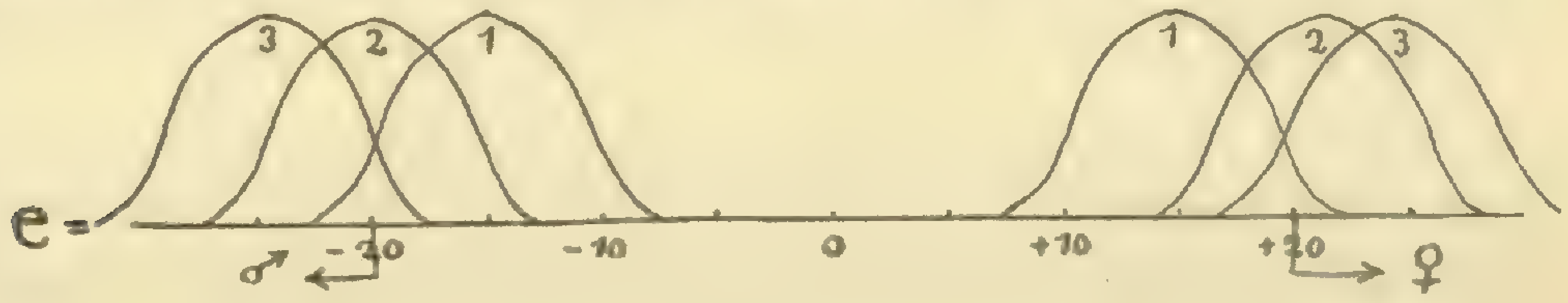

FIG. 3

approach the epistatic minimum (20 and —20 in our diagram), then a point must be reached where the variability-curve stretches beyond this limit. That means—curve 1—that in cases of low grade intersexuality some + or — individuals might overlap into the normal. Of course any position of these curves and a corresponding numerical relation of normals and very slightly intersexual animals might be met with, as the curves 2 and 3, Fig. 3, indicate. And indeed such cases have been found as well on the female as on the male side. Types of this kind, realized in the F_2 crosses with male intersexuality, are those with which we have dealt in the previous paragraph. Thus far all facts agree with this conception, in one culture up to F_5. Further checks are in progress.

But there is another possibility for the production of intersexual males. Let us assume that we could find two races of the following constitution:

Strong race ♀ =	FF	Mm	♂ =	FF	MM,
	100	60		100	60 60

Weak race ♀ = [FF] Mm ♂ = [FF] MM.
80 50 80 50 50

The F_1 cross of these, using the strong race as mother, would give:

F_1 ♀ [FF] Mm = normal female,
100 50

F_1 ♂ [FF] MM = intersexual male, as e = — 10.
100 60 50

Every single male ought to be intersexual. Through the kindness of Dr. Machida in Tokyo, who first has reached this strange result, I could bring back from Japan two such races and they breed exactly according to expectation.

This is about the skeleton of our work around which of course many interesting experimental, morphological, embryological, cytological and physiological facts are grouped. I am rather optimistic in regard to the general conclusions, which might be drawn from these facts as well as regards the sex-problem as on some fundamental questions of heredity. Combining these facts with the work on hormone action as related to sex we can, I think, form a pretty clear idea about sex-differentiation and determination.[9]

If we put them in line with the facts of experimental embryology concerning the determination problem we see the outlines of a promising theory of heredity. But this can be discussed only when a full account of the work has been given.

[9] The relation of gametic or zygotic intersexuality to the hormonic intersexuality produced in castration and transplantation-experiments in Crustacea, birds and mammals (especially the work of G. Smith and Steinach) to which now, after F. Lillie's discoveries, the case of the free-martin has to be added, has been worked out in the chapter on Sex in my treatise on genetics (*l. c.*), 2d edition. A detailed comparison between the moth-work and the facts known about human intersexuality, containing, too, a discussion of the relations of zygotic and hormonic intersexuality, was sent to Germany for publication about half a year ago, but it is not known whether it has been published there or adorns the waste-paper basket of His Britannic Majesty's censor.

PIEBALD RATS AND MULTIPLE FACTORS

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Introduction

The experiments of Castle and Phillips (:14) with piebald rats afford the largest mass of recorded data on the influence of selection in mammals. For 17 generations, the area of pigmentation on their hooded rats has been increasingly modified. In one line (the plus race) the pigmentation has been extended; in the other line (the minus race) it has been reduced. When rats from the plus or minus race are crossed with fully pigmented rats, such as the normal wild, or the Irish variety, the hooded pattern behaves as a simple Mendelian recessive, disappearing in the first generation and reappearing in one fourth of the offspring in the second generation. These results lead to the conclusion that hoodedness appears when a certain germinal unit, or factor, is in a zygote in a homozygous condition. Besides this, Castle concludes that the factor determining hoodedness fluctuates, and, in accord with its fluctuations, the amount of hooding varies. It follows that the selection of extreme grades of hoodedness results in the simultaneous selection of extreme variations of the factor. Moreover, Castle (:16, p. 722) finally concludes that the selection of these extreme grades of hoodedness influences the direction in which the factor for hoodedness varies.

These conclusions bear on one of the most generally interesting and vital questions before biologists. If, besides deciding which individuals shall mature and reproduce, selection can influence the direction in which the units of inheritance, or factors, vary, there can be no question but that

selection, as an agency in evolution, must then be restored to the important place which it held in Darwin's estimation, an agency capable of producing continuous and progressive racial changes (Castle, 15*b*, p. 97).

Castle's experiments have justly become famous. For eight years they have been continuously in progress; they have involved large amounts of arduous labor; they have been conducted with unflagging zeal and high ideals of scientific attainment. The conclusions drawn from such an important investigation should receive painstaking consideration.

The writer has been conducting selection experiments which have led him to conclusions different from those reached by Castle. Although these experiments have not involved the expenditure of so much labor and time as did Castle's work, they include three times as many generations and four times as many individuals as are reported by Castle. One investigation was on rats, the other on flies, yet there are so many similarities in the results that the writer was led to make a careful analysis of Castle's papers in an attempt to discover the basis for the conflicting conclusions. The final result of this study was to make the writer feel that the following statements in regard to the hooded rats are too positive.

All the evidence we have thus far obtained indicates that outside modifiers will not account for the changes observed in the hooded pattern, itself a clear Mendelian unit (Castle, :15*b*, p. 722).

. . . there can be no doubt that only a single genetic factor is here involved (Castle, :16, p. 95).

It is precisely this last named category of cases [a single factorial basis undergoing quantitative variation] which alone can explain our rat results (Castle, :15*b*, p. 725).

Energetic attacks have been made on the interpretation Castle has given to his results, and certain unwarranted criticisms have been duly answered. That the theory of multiple factors may be applied to the results as published in 1914 in the Carnegie Institution Publication No. 195 was indicated therein by Castle, and further emphasized by Muller (:14). Most of the criticisms of the experiments with the hooded rats have been based on the

generalizations that have been made, and not directly on the data. In this paper the writer has used the original data, making verifications where possible, and recalculations of many of the constants. A few inconsistencies and arithmetical errors were found.

That there may be no misunderstanding as to the nature of the multiple factor interpretation, the following scheme is suggested. In the absence of any factor that determines uniform color—in other words, in the presence of two doses of the factor for hoodedness—the amount of pigment on hooded rats may be influenced by several factors. Some of these increase, others reduce, the pigmented area. The factors that increase the pigmented areas (plus factors) form Mendelian pairs (allelomorphs) with the factors that decrease the pigmented areas (minus factors). Dominance is lacking; if a factor is contributed to the zygote by both parents, that factor has more power than if it had come from only one parent. Furthermore, environment, or other conditions which are not inherited, being outside the germ plasm, have such a modifying influence on the pigmented areas that the potential differences between individuals determined by different combinations of factors in the germ plasm, are frequently concealed. It is not pretended that this is the only application of the multiple factor hypothesis that can be made, but it is hoped that the following arguments may become more significant with this suggested application in mind.

The writer herein undertakes to show that the conception of multiple factors may still be applied to Castle's data. The points that favor the multiple factor interpretation of the rat experiments, as well as certain objections that are said to definitely disprove this theory, are brought together in the following paragraphs.

Points Favoring the Multiple Factor Interpretation

1. The gradual divergence of the plus and the minus races may be brought about by the sorting out of groups

of different factors. It has been generally recognized that this is a possible conception. The following authors have considered this point: Castle and Phillips, :14, Muller, :14, Hagedoorn, :14, MacDowell, :15.

2. The hybrid ancestry of the original parents affords a source for a large amount of heterozygosis. It is the reduction of this heterozygosis that selection is supposed to accomplish, in separating the two races, plus and minus.

3. Such a reduction of heterozygosity would be hindered by the large number of matings made between rats less closely related than brother and sister. This point has been discussed by Muller.

4. By breaking the correlation between the soma and the germ plasm, environment has probably played a large part in hindering the reduction of heterozygosity. Apparently it has been assumed that there is a close relationship between the germ plasm and the soma, that the smooth curve of the averages in successive generations proves that the germinal variations, to which the rise in the curve is due, are small and constantly occurring. But, since the rôle of environment is not known, the gradual advance in the averages can not prove anything as to the size of the germinal variations. The presence of regression makes it clear that environmental, or extra-germinal, influences are active in producing variability in the hooded pattern. Regression is really a measure of the degree of independence of the soma and germ plasm. Regression expresses the inverse relationship between the actually tested breeding possibilities and the appearance of the parents. There can be no question as to the activity of environmental influences; of their power and nature nothing seems to be known. The immediate environment of the undifferentiated blastomeres is probably as important a factor in the final appearance of a character as the germ plasm itself. The factors in the germ plasm are like chemicals that will react in a definite way in connection with certain other chemicals; when

different ones are combined with the first ones, the results may be reversed. Now to study one variable (germ plasm) through a measure (soma) influenced by a second variable (environment) will seldom give correct results if the effect of the second variable is not clearly recognized and discount made for its influence. In the present case, it appears that the curve of the averages can only show the degree to which the variations due to environment and the germinal variations tend to go in the same direction. That there is a rise in the curve shows that, on the average, they are a little more likely to agree in direction than to contradict each other. On the other hand, since the environmental variations can not be accounted for and eliminated, the curve gives no information as to the actual or relative potencies of either set of variables. That there are no fluctuations in the curves may have been assumed to prove that environment is constant and therefore does not demand consideration. But this conclusion can not be safely drawn from the facts. The curves probably do mean that, when generation is compared with generation, the variations of the environment are cancelled out; they mean that these environmental, or extra-germinal, variations occur within a generation, and probably within a family or within the gonads of the parents. Environment might well be ignored were the ultimate question to be answered, "How much can selection change the average grade of hooded rats?" But this is not the main question. The question to be answered is, "What is the nature of the changes in the germ plasm?"

In view of all this, one can find slight justification for assuming that the germinal variations were small and constantly occurring. It seems entirely possible that the environmental, or extra-germinal influences were strong, perhaps even more effective than the germinal constitution. In this case, there would be no need to assume a very large number of factors to find a multiple factor explanation for the slow advance wrought by selection.

Such strong environmental influences would, for the most part, effectively confuse the various combinations of germinal factors, and selection would continue to produce slight advances for a long time.

5. Castle has explained (Castle and Phillips, :14, p. 24) the significance of the ". . . observed reduction of variability" for the multiple factor interpretation; he stated at the same time that ". . . extensive modification through selection is possible without any marked falling off in variability." Since the observed reduction

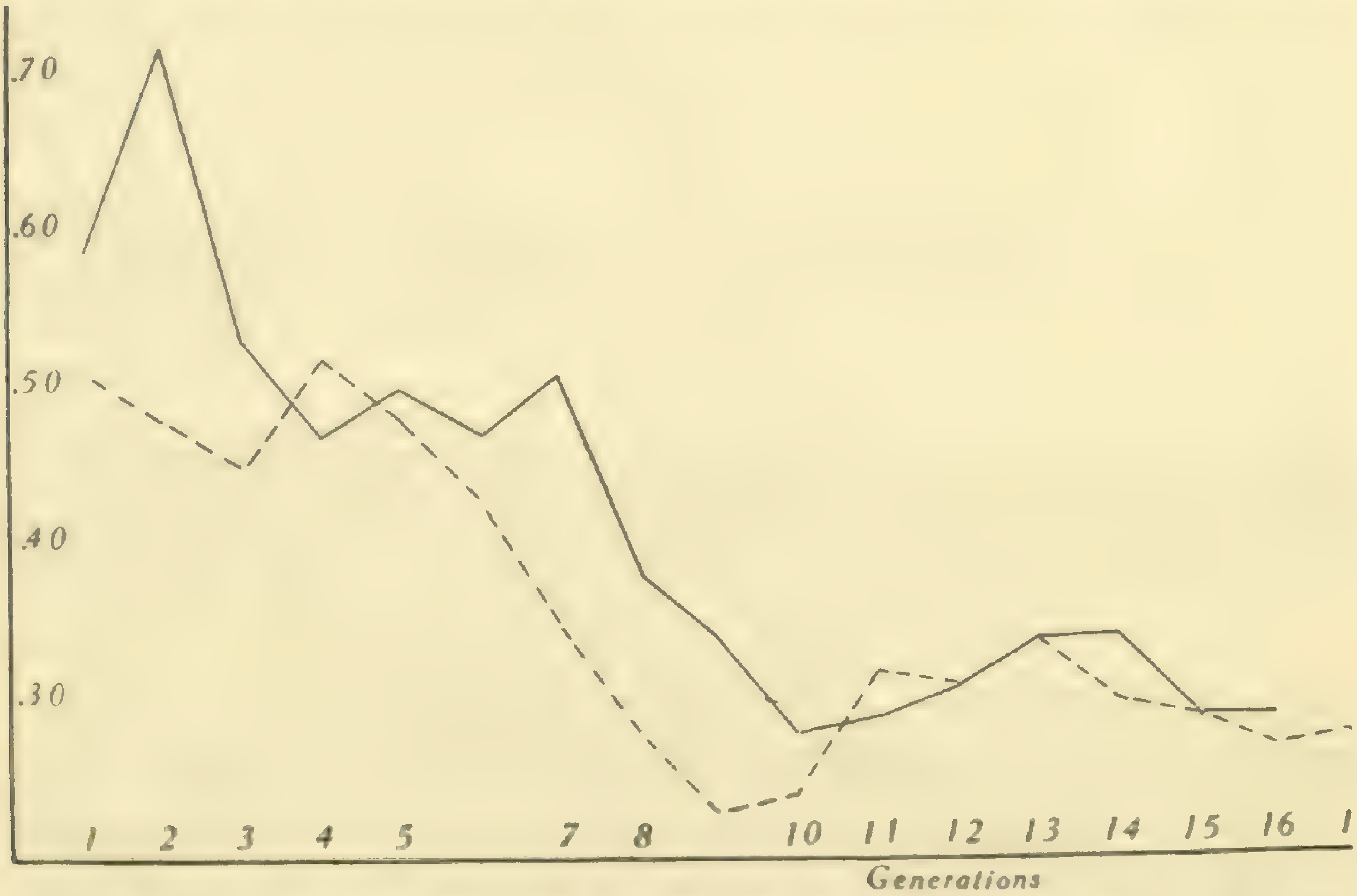

FIG. 1. Standard deviations of the plus race (solid line) and minus race (broken line) in the various generations of selecting. Ordinates represent standard deviations in terms of grades of hooding; abscissæ represent generations of selecting. Calculations made from the data as given by Castle and Phillips, 14, in Tables 1–13 and 16–28.

in variability is not considered to be marked, Fig. 1 is presented to show the facts graphically. The standard deviations plotted in this graph have all been calculated directly from the data, and in several cases they differ slightly from those given by Castle. The decrease in variability that is shown by this figure is the expected result of reduced heterozygosity accompanying continued selection.

6. The question of the rate of advance has been an-

swered by the statement that "no slowing up is observable in the rate of change of the racial character under selection either plus or minus" (Castle, :16, p. 96). This is assuredly a very vital point in the contention that multiple factors will not explain the results. For, if the rate of advance has not fallen off, and if, during seventeen generations, each selection has been as effective as the preceding one, it certainly would look as though this progress were due to constantly varying germ plasm, and not to the sorting out of certain groups of factors. Were a sorting out of factors going on, each advance would restrict the possibilities for further advances, so that in a series of selections the rate of advance would decline.

In Castle's "Heredity," page 122, Fig. 41 are shown the curves of the averages of the first eight generations of the plus and minus races. These curves begin with the average of the offspring that appeared after the first selection. From this point on, the advance shown by the curves is gradual. But should not the advance resulting from the first selection be recorded? The average of the first selected generation was not the point of departure. To show the advance resulting from the first selection, the first point of the curve must give the average of the hooded race before the first selection. Unquestionably the difference between the average of the unselected race and the first selected generation was an advance due to selection, yet this advance is apparently ignored in the statement quoted above, as well as in the figure cited. The first selection resulted in a very much greater advance than any other single selection in the whole series. It took the ten subsequent selections to separate the means of the two races as far as the first selection separated them. If each selection had produced a like advance, the eleventh generation of selection should find the averages of the two races eleven times as far apart as they were after the first selection instead of twice as far apart. Failure to consider the advance due to the first selection has concealed one of the most striking features of the

whole series of experiments, namely, that the first selection brought about an immediate and abrupt establishment of two races with means 3.05 grades apart. The greatest divergence between the two races due to a single selection in all the following generations was 0.64 grade. This followed the third selection. In the second generation there was a reduction of the average of the plus race. Castle explains this as follows:

> To obtain larger numbers of offspring, several new pairs were added to the experiment in this generation which did not appear in Table I either as offspring or parents, but which were derived from the same general stock as the parents of generation one (Castle and Phillips, :14, p. 9).

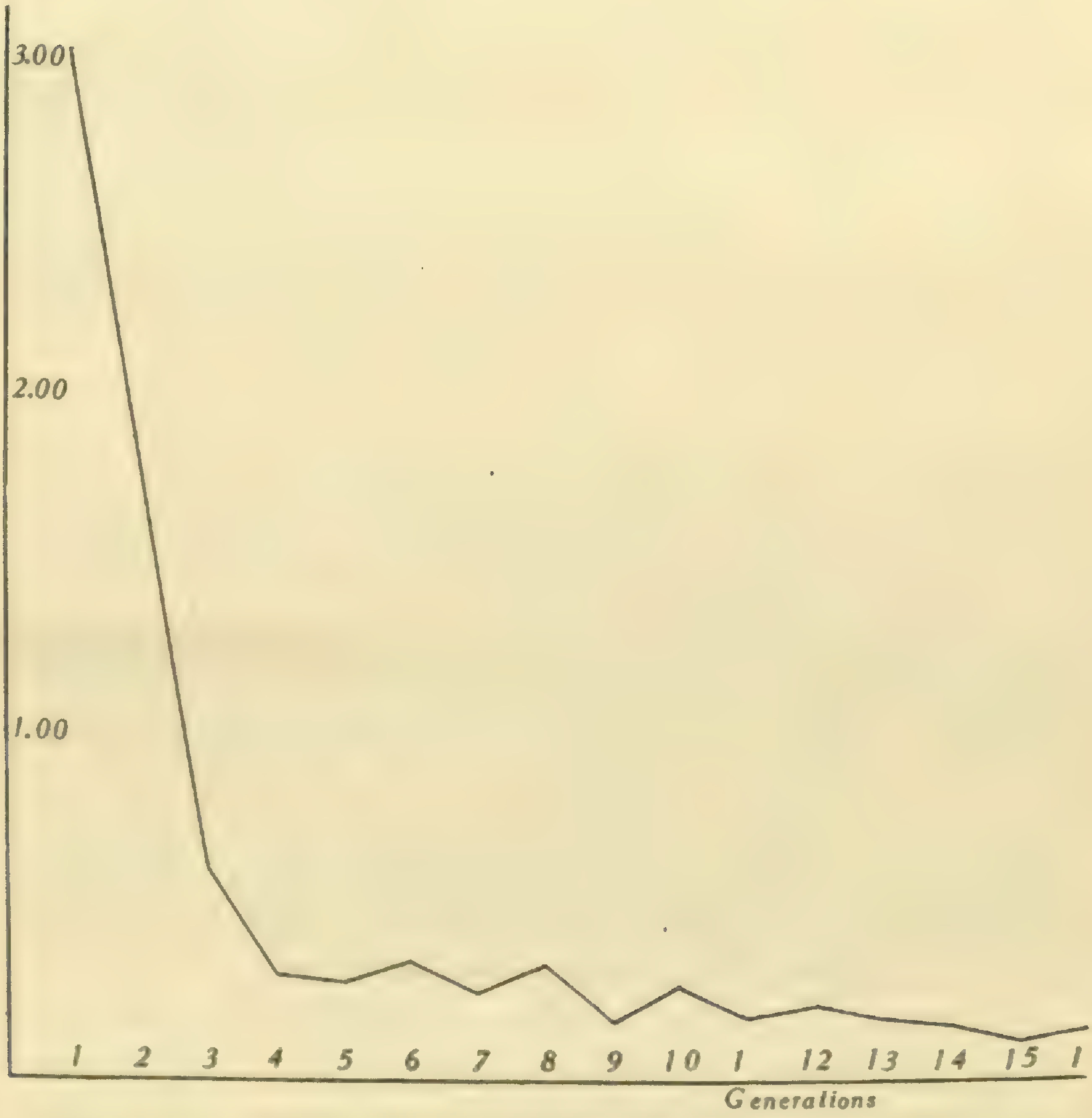

FIG. 2. Rate of divergence of the means of the two selected races in the various selected generations. The point for the second generation has been omitted from the curve: see explanation in text. Ordinates represent grades of hooding: abscissæ represent generations of selecting.

After the third generation there is, in general, a gradual decline in the effectiveness of selection, till in the fifteenth generation the advance is 0.12. In support of this statement, which stands in direct disagreement with the quotation at the head of this section, Fig. 2 is offered. In this figure the ordinates represent the increases in the differences between the two racial means in successive generations. Since the decline in the second generation, as explained, seems to have no immediate significance, this point has been omitted from the curve. The advance in the third generation has been calculated from the difference between the two races in the first generation, which of course gives a slightly smaller advance than if the difference in the second generation had been used. The greater part of the falling off occurs in the plus race, but both races show the same general tendencies, namely, a sudden advance as the result of the first selection, with much reduced advances following subsequent selections. It may be supposed that there was a greater degree of heterozygosity in the parents selected to start the minus race than in those selected to start the plus race. This might explain the smaller initial advance in the minus race (one unit as compared with two in the plus race) as well as the more prolonged and slower subsequent advances.

7. Regression, as Castle uses the term, is the difference between the averages of the selected parents and their offspring. It is due to the imperfect correlation of two variables, intra-germinal and extra-germinal differences, and so, as stated, it forms a gauge of this relationship. Its amount will be reduced by a reduction in the amount of variability of either variable. In the later generations the regression is reduced. We see no reason to suppose that environment as a whole acts any differently in different generations. Therefore this reduction in the amount of regression becomes further evidence in support of the supposition that the germ plasm is more uniform, more homozygous in the later generations.

8. As Muller has correctly reasoned, successful return selections would be expected on the multiple factor view, supposing the races were still "heterozygous even after generations of selection" (Muller, :14, p. 571). It may be added (MacDowell, :15, p. 95) that the failure of a return selection to reduce the average, as long as the advance selection was progressing, would be strong evidence against the multiple factor interpretation. As long as there remained any heterozygosity in the race, both advance and return selection should succeed in moving the averages.

9. In no case should return selection lower the averages at a rate faster than advance selection was raising them at the same time. That is, a certain degree of heterozygosity will permit a certain rate of advance or decline of the averages. The return selections that were started after several advance selections did not show a decline that could be compared with the sudden advance that occurred in the first selected generation. The plus selections had reduced the heterozygosity and had thus set closer limits on the effectiveness of return selection, as well as of further advance selection. So return selections from later generations should be less effective than return selections from the earlier generations. In the plus race, generation 7, there is a difference of .84 between the average of the offspring of rats selected to continue the plus race and the average of the offspring of rats chosen for a return selection. In generation 12 the corresponding difference is .60. In the minus race the difference between the averages of offspring from high and low grade parents in 7, 8 and 9 average .50. In generation 12 the difference is .28. The numbers of rats are very small in most cases, but it is interesting to note that as far as they go they seem to show that return selection is less effective in the later generations than in the earlier ones.

10. Castle has shown that the increase in variability in the first generation of a cross between the plus and minus races may be considered an indication of segrega-

tion, therefore, of heterozygosity in the races (Castle and Phillips, :14, p. 30). Muller (:14, p. 573) repeats Castle's suggestion that the further increase in the variability in the second generation of this cross argues for the supposition that the two races differ in regard to several factors. This is a logical interpretation uncontested by the facts, though of course it is not proved. On the other hand, it has been proved, by the crosses between the two races, that they are not distinguished by just one Mendelian unit. Now if the increased variability in the F_1 of the cross between the plus and the minus races be due to heterozygosis in these races, and if selection is reducing this heterozygosis, crosses made after the races had been selected for a longer time should give less variability in F_1. The figures show this to be the case. If there is less variability in F_1, in later generations there should correspondingly be less variability in the F_2. We find:

F_1 from cross plus by minus, generations 5 and 6 = S. D. 0.71; generation 10 = 0.60

F_2 from cross plus by minus, generations 5 and 6 = S. D. 1.01; generation 10 = 0.87

11. The reductions in the averages of both the plus and minus races after crossing with wild or Irish, first led Castle to consider a factorial interpretation (Castle and Phillips, :14, p. 25). Muller (:14, p. 574) has fully restated the bearing of this on the multiple factor theory. The cross has apparently undone selection to some extent by restoring some of the factors that had been selected out in forming the two races; the cross has increased the heterozygosity of the extracted hooded rats, returning plus factors to the minus race and minus factors to the plus race.

In the light of the above interpretation, the conversion of the minus race into the plus race by means of a cross is significant. Selection for increase in pigmentation was started from extracted hooded rats from a cross of minus with wild. The first generation of this selection made as sudden an advance as the first generation of selected plus rats did at the beginning of the experiment.

It is to be observed that a cross makes a profound difference in the effectiveness of return selection. Crossing has so modified the germ plasm that rats from the minus race immediately, without any gradual return to the "0" grade, repeated the history of the plus race. Further, plus selection was carried on in this new race. Castle (Castle and Phillips, :14, p. 21) emphasizes the fact that this race is free from the objection urged against the main experiment, namely that the closest inbreeding was not carried out. Further interest in this closely inbred race lies in the fact that, although it starts out with a curve almost identical with the first generations of the plus race, the rate of advance falls off faster than it does in the main plus race. One may suppose that the cross produced an F_2 in which some rats had a degree of heterozygosity similar to that which existed in the original unselected stock; a closer inbreeding reduced the heterozygosity more rapidly.

12. The earlier generations of the plus race when crossed with wild are only slightly reduced in pigmentation. In Table 43, Castle and Phillips (:14, p. 48) show, among other things, the averages of hooded grandchildren extracted from a cross with Irish. In comparison with these are placed averages specified to be of offspring from the same grade parents and the same generation of the uncrossed selected race. References to the proper tables of the uncrossed selected races show practical agreement with the averages as quoted in this Table. In Table 42, which gives corresponding results of crosses with wild, three of the averages of the uncrossed races are taken from the same generation as the parents crossed, and three seem to be taken from the following generation. It is a matter of importance to have correct standards for judging the modifications due to crossing. There might be a question whether one should use the average of the generation from which the parents came, or the following one; but in either case the use should be constant. Although the averages of

the generations from which the parents came have been used for comparison in eight of eleven crosses, it appears to be a more fair procedure to compare the averages of the offspring produced by parents of the same grades and generations as used in the crosses. Suppose the hooded parent crossed was grade 2, from the fourth generation, then the average of the *offspring* from parents of grade 2 from generation 4 should be compared with the hooded offspring in F_2. In other words, the average to be used for comparison would be found in generation 5. On this basis the comparisons shown in Table I have been made. Returning now to the statement at the head of this section, that when crossed with wild, the earlier generations of the plus race are only slightly reduced in pigmentation, this table shows that, when third generation parents were crossed, the extracted hoodeds were lowered .04; when fifth and sixth generation parents were crossed, the lowering of their hooded grandchildren was greater, .17; when the hooded parent came from the tenth generation, the average of the hooded grandchildren was lowered .76.

TABLE I

CALCULATION MADE FROM DATA FROM CASTLE AND PHILLIPS, :14, TO SHOW THE EFFECTS OF CROSSING ON THE AVERAGES AND STANDARD DEVIATIONS OF THE EXTRACTED HOODEDS

Numbers in brackets are those given by Castle and Phillips.

A. Comparisons of the Averages of Extracted Hooded Rats with the Averages of the Offspring of Hooded Rats of the Same Grade and Generation as the Hooded Rats Used as Parents in the Various Crosses.

	Wild by Minus Race		
Generation from which hooded parent came	2½	6	10
Average grade of F_2 hoodeds	+ .31	+ .25	+ .25
Average grade of uncrossed hoodeds	— 1.18	— 1.72	— 2.12
Average grade of uncrossed hoodeds as published	(— 1.20)	(— 1.59)	(— 2.05)
Raised by cross	1.49	1.97	2.37

	Wild by Plus Race		
Generation from which hooded parent came	3	5 + 6	10
Average grade of F_2 hoodeds	+ 2.56	+ 2.97	+ 3.15
Average grade of uncrossed hoodeds	+ 2.60	+ 3.14	+ 3.91
Average grade of uncrossed hoodeds as published	(+ 2.60)	(+ 3.14)	(+ 3.84)
Lowered by cross	.04	.17	.76

	Irish by Minus Race		
Generation from which hooded parent came	3½	4	7½
Average grade of F_2 hoodeds	—.62	—.73	—.94
Average grade of uncrossed hoodeds	—1.28	—1.64	—1.83
Average grade of uncrossed hoodeds as published	(—1.31)	(—1.18)	(—1.75)
Raised by cross	.66	.91	.89

	Irish by Plus Race	
Generation from which hooded parent came	2	3
Average grade of F_2 hoodeds	+1.27	+.95
Average grade of uncrossed hoodeds	+2.10	+2.60
Average grade of uncrossed hoodeds as published	(+1.80)	(+2.50)
Lowered by cross	.83	1.65

B. Comparisons of the Standard Deviations of Extracted Hooded Rats with the Standard Deviations of the Offspring of Hooded Rats of the Same Grade and Generation as the Hooded Rats used as Parents in the Various Crosses.

	Wild by Minus Race		
Generation from which hooded parent came	2½	6	10
S. D. of F_2 hoodeds	1.03	.90	1.18
S. D. of uncrossed hoodeds	.56	.33	.31
S. D. of uncrossed hoodeds as published	(.49)	(.44)	(.24)
Increased by cross	.47	.57	.87

	Wild by Plus Race		
Generation from which hooded parent came	3	5 + 6	10
S. D. of F_2 hoodeds	.50	.52	.45
S. D. of uncrossed hoodeds	.47	.47	.29
S. D. of uncrossed hoodeds as published	(.53)	(.49)	(.36)
Increased by cross	.03	.05	.16

	Irish by Minus Race		
Generation from which hooded parent came	3½	4	7½
S. D. of F_2 hoodeds	.64	.60	.84
S. D. of uncrossed hoodeds	.53	.34	.26
S. D. of uncrossed hoodeds as published	(.48)	(.46)	(.35)
Increased by cross	.11	.26	.58

	Irish by Plus Race	
Generation from which hooded parent came	2	3
S. D. of F_2 hoodeds	.90	.87
S. D. of uncrossed hoodeds	.38	.47
S. D. of uncrossed hoodeds as published	(.75)	(.53)
Increased by cross	.52	.40

If the difference between these selected generations lies in the changed position of the mode of continuous ger-

minal fluctuations, one would have difficulty in accounting for the above facts. If these various selected generations differ in the number of multiple factors they bear, one can easily understand that the reason that practically no modification is apparent when the third generation is crossed, is that the number of plus factors in this generation and in the wild are not very different; in the fifth and sixth generations there may be a few more plus factors than in the wild, and in the tenth generation there are several more.

13. The early generations of the plus race, although only very slightly lowered by crosses with wild, are strikingly lowered by crosses with Irish. In a cross in which the hooded parent came from the second generation, the lowering was .83; when the hooded parent crossed came from the third generation, the lowering was 1.65. Now how may this fact be interpreted? If the change in the means following a cross be assumed to be due to the action of different numbers of factors in the races crossed, it is clear that this particular wild is more like the plus race in regard to its factors than is the particular Irish race. In other words the wild race seems to have more plus factors than the Irish race. When early generations of the plus race are crossed with wild there is hardly any change in the averages of the F_2 hoodeds, because there are about the same plus factors in the wild as in these early generations of the plus race. When these same generations of the plus race are crossed with Irish there is a considerable decrease in the averages because there are fewer plus factors in the Irish than in the early generations of the plus race. Now if the germ plasms of the wild and Irish differ in regard to the number of accessory factors, and if the germ plasms of the plus and minus races differ in this same regard, comparisons of all the crosses between these races should show the following results: crosses between wild and minus should give greater modifications in F_2 than crosses between wild and plus; crosses between Irish and minus should modify the

F_2 hoodeds less than crosses between Irish and plus. More directly, the plus race should be more modified by the Irish, the minus race more modified by the wild. Observation of Table I will show that these results are realized.

As already noted in the case of crosses between the plus and wild races, this table shows that in other crosses the different generations of the selected races are differently modified. After long selection there is more modification as the result of crossing. This generalization is supported by all the averages and all the standard deviations in crosses involving the wild race; it is supported by all but one average and by all but one standard deviation in crosses involving the Irish race. If selection is sorting out different groups of factors in the plus and minus races, crosses made after many selections bring together groups of factors more diverse than when crosses are made after only a few selections. The greater the diversity in the numbers of plus or minus factors in the animals crossed, the more extensive will be the segregation in the second generation. More extended segregation may be expressed by increased variability and by more pronounced modification of the averages of the F_2 hoodeds.

14. The behavior of the "mutant" in crosses with the plus and minus races gives clear support to the multiple factor hypothesis. Castle (Castle and Phillips, :14, p. 29) has clearly demonstrated this point. The "mutant" is a suddenly appearing, quantitatively increased stage of the hooded character, that is controlled by a Mendelian factor. Crossed with the race from which it sprang, the extracted individuals show no change from the uncrossed race, either as to averages or variability; crossed with the other race, modifications were found, equalling those obtained when the two races were crossed together. The newly discovered factor acts independently of the other factors, is not modified by them, and does not modify them. Being the one difference between

the mutant and the plus race at the time the mutant appeared, this factor affords a critical test for the interpretation of the modifications that result from crosses.

Objection to the Multiple Factor Interpretation

One new point since 1914 has been urged against the application of the multiple factor hypothesis to the results. By the strength of this evidence the authors of the rat publication are "forced to conclude that this unit (hoodedness) itself changes under repeated selection *in the direction of selection*"; (Castle, :15*b*, p. 722). The point follows:

> The changes effected by selection show permanency under crosses with wild rats. They change no more nor less than an unselected hooded race does. A first cross of the selected race seems to show a partial undoing of the changes produced by selection, but a second cross made on a still larger scale, involving over 1,000 second-generation individuals, showed no further change of this sort, but instead a return to about what the selected race would have been had no crossing at all occurred (Castle, :16, p. 96).

If the grade of hooding of the plus race is reduced in crosses with wild by the replacement of factors selected out of the plus race, repeated crossing of the modified rats should produce further reduction. On the basis of the above claim that crosses do not produce such modifications in the hooded pattern all the evidence formerly admitted to favor the multiple factor interpretation has been swept aside. No one would claim that a single strongly supported experiment may not upset large amounts of contrary evidence, but in such cases it is of utmost importance to have the validity of the crucial experiment fully supported. Is the claim that crosses do not change the selected races fully supported? The following are all the data we are given on this point:

Extracted hoodeds from
hooded × wild ....75 rats, average 2.89; regression on grandparents .56
Extracted hoodeds from extracted
hooded × wild ....263 rats, average 3.33; advance on grandparents .32

Averaging the 75 hoodeds may first be criticized. These include all the extracted hoodeds that came from crosses between the wild and the plus races. The third, fifth, sixth and tenth generations of the plus race are involved. It has been shown that the early generations of the plus race are not lowered very much by the crosses in comparison with the tenth generation, which was considerably modified. Therefore among these 75 extracted hooded rats are some that were lowered by the crosses, but more that were practically unmodified. Moreover, the 263 twice extracted hooded rats came from ancestors that had been selected for at least ten generations. Only 16 of the 75 once extracted rats had ancestors that had been selected for ten generations; the others, having ancestors selected for a shorter time, would be expected to give lower averages. In testing for further lowering in this second cross it would seem unjustified to use an average including rats not lowered by the first cross or rats that had not been selected for an equal number of generations before the crosses. Modified by the above considerations the comparisons stand as follows:

Extracted hoodeds from tenth gen. plus × wild	**average 3.15**
Extracted hoodeds from extracted hoodeds × wild	**average 3.33**
Uncrossed, same generation and grade as hooded grandparent	**average 3.84**

The conclusion has been quoted that the cross of the extracted hoodeds with wild has not carried on a further reduction, but it has shown a return, "to about what the selected race would have been had no crossing at all occurred." Will the above figures support this conclusion? The cross of the extracted hoodeds with wild does indeed give a higher F_2 average than the cross of the tenth generation, but the difference is only slight (.18). These two averages are based on very different numbers. It is entirely possible that a larger number of rats extracted from the first cross would have had a higher average than that of the rats extracted from the second cross; in such

a case the second cross would be said to show further reduction.

Whether this advance in the second cross returns the hooded grade to about what the uncrossed race would have been is a matter of what average is used to represent the uncrossed race. The original hooded parents were the last parents to be selected in this series of crosses. It seems clear then, as above reasoned in another connection, that the average to be used in comparison with the two groups of F_2 hoodeds is that of the *offspring* of uncrossed parents of the same grade and generation as the original hooded parents used in the crosses. If this average be accepted (3.84), it is plain that even after the second cross there remains a considerable difference between the averages of the uncrossed and the twice extracted hooded rats. There is reason to believe that the changes produced by selection *are* modified by crossing and that it has not been finally disproved that further crossing does not cause further modification. So, as far as can be judged from the data at hand, this crucial test does not seem to offer a final blow to the applicability of the hypothesis of multiple factors.

On the other hand, that modification actually does result from crosses is strikingly proved by the conversion of the minus race into the plus by means of a cross. This experiment has been referred to on page 729. Six successive return selections did not return the average of the minus race to the "0" grade. But after minus race rats were crossed with wild, a single selection of the plus varieties raised the average 2 grades above "0."

Summary

By way of recapitulation, the points referred to are summarized as follows:

A. Seventeen generations of selection need not have entirely eliminated modifiers, because,

1. Matings less close than brother and sister have tended to continue heterozygosity;

2. Environmental influences may possibly act in such a way that only occasionally does a selected individual carry germ plasm more homozygous then the average.

B. The implied claim that the facts do not support the supposition that selection has decreased the number of modifiers, or has reduced the heterozygosity in the two races of rats, has been answered by the following points:

1. Selection reduces the variability.
2. The rate of advance declines as selection is continued.
3. Parental regression is lowered by selection.
4. Return selections argue that heterozygosis is still present; they indicate that there is less heterozygosis after longer selection, since selection reduces the effectiveness of return selections.
5. Crosses between the plus and minus races strongly suggest that heterozygosity is still present by the increase in variability in F_1; they also appear to show that there is less heterozygosity in a later generation, since the increase in F_1 is less in a cross after longer selection.
6. Crosses between the selected races and the wild or the Irish race show that more modification appears in the F_2 hoodeds when crosses are made after longer selecting.

The reader is now in a position to judge whether the writer is justified in concluding that there is still a "possibility that other as yet undiscovered factors might be responsible for the apparent changes observed" (Castle, :15, p. 722) and that the claim that "all the evidence we have thus far obtained indicates that outside modifiers will not account for the changes observed" is too sweeping.

Discussion[1]

A great difficulty has been placed on the discussions of this subject by the different terminology used by those holding different opinions. Calling the visible character the Mendelian unit is a striking example of this difficulty. There is a vital difference between a unit character and a factor, which must be constantly recognized if this discussion is to progress.

It is unfortunate that the word *selection* has come to have the significance of a slogan. For the nature of the actual power of selection itself is not in question. What selection is, can be easily defined and agreed upon. If the nature of the changes in the germ plasm could be determined, there would be little disagreement as to what selection could accomplish. Even those who are not considered to be selectionists believe that natural selection is very important in evolution. So the epithets, selectionist and pure-lineist, fail to indicate the difference between the two groups to which they have been applied. It would be quite impossible to divide biologists into two distinct schools on the basis of a subject upon which there are many different shades of opinion. Any such classification would be inaccurate, even if the most precise definitions of the classes were generally accepted. When there are no accepted definitions, and those most clearly cut are offered by individuals in the opposite group (each one realizing the diverse ideas within his own group and wishing to crystallize an opposing view in order to attack it) such classification of opinion is far from scientific. In the present instance, the classification into selectionists and pure-lineists has tended to magnify the differences between investigators. With a desire to try to overcome

[1] Since the writing of this paper, there have appeared papers by Pearl ("Fecundity in The Domestic Fowl and The Selection Problem," Amer. Nat., 1916, p. 89) and Castle ("Can Selection cause Genetic Change?" Amer. Nat., 1916, p. 248) which have a close relationship to the present discussion. It has been considered wiser to leave this paper as written, than to enter the controversy by including discussions of the two papers mentioned.

the exaggerated differences which seem to exist, the following discussion is offered. It is written with no wish to codify or defend the opposing positions, but rather as an attempt to formulate the issue a little more clearly by presenting two views, which appear to have advocates, of the nature of the changes in the germ plasm.

The view to be called the "first" is as follows: The changes in the germ plasm are in the nature of fluctuations, now larger, now smaller, but continuously appearing; they lead in all directions. This is true of all inheritance, whether or not it be factorial (Mendelian) in basis. If it refers to Mendelian inheritance the potential grade of the factor in question, as found in any zygote, acts as a mode about which the fluctuations in potentiality occurring in the next generation are grounded. In other words, although a zygote may include the strongest potential grade of a factor that has appeared, the inevitable fluctuations in this factor that are found in the different gametes formed by this zygote will include, together with those like and weaker than the parent, some with stronger potentialities than the parent.

The view to be called the "second" is as follows: The changes in the germ plasm are discontinuous; they appear fortuitously. They may strike out in almost any direction, as a projectile may be aimed in "any direction," in contrast to the "all directions" taken by the waves of sound when the projectile explodes.

According to the first view, selection would result in modification in any direction the breeder might desire, irrespective of variational tendencies shown by the animal. To maintain conformity to type would require as constant selecting as would be required to obtain divergence. According to the second view, selection could progress only in certain directions, depending on how the germ plasm happened to change; the variational tendencies of the animals would probably suggest these directions. Conformity to type would be considered to be a fundamental phenomenon due to the conservative tend-

ency of the germ plasm to maintain the *status quo*. On the basis of the first view, the external influence (selection) would have major importance in defining the course of evolution; on the second, the internal influence (the inherent nature of the germ plasm itself) would have major importance. In both cases, the nature of the progeny would depend on the nature of the germ plasm of the parents. In both cases selection would be able to modify the race. But in neither case is the origin of the changes in the germ plasm explained. The fundamental causes of evolution are as much a mystery as ever. Grant a certain hypothesis of germinal changes, and selection becomes a more important factor in evolution than when another hypothesis is granted. But even such an increased importance of selection does not give it the value of a fundamental creative cause of evolution.

There has appeared a theory that would give selection still greater importance by saying that selection has the power to build up unit-factors and induce mutation.

> Unit-characters may arise gradually as the result of repeated selection in a particular direction (Castle, :12*b*, p. 280).
>
> In yellow animals, as in blacks, individuals of varing intensity occur the darkest known as reds, the lightest as creams. A complete series of intermediates can be obtained if so desired. If we select any two widely separated stages in this series fairly stable in their breeding capacity and cross these, they Mendelize, *i.e.*, they behave as if they were a single unit-character difference between them. . . . That difference might equally well be *half* as great as it is, or a *quarter* as great, or a thousandth part as great. A monohybrid ratio would result equally in each case, upon crossing the two quantitatively different stages (Castle, :12*a*, p. 358).

Now this may be true for yellow guinea pigs, but the rats clearly demonstrate that it is not true in all cases. The two quantitatively different stages of the hooded pattern represented by the plus and minus races do not result in a monohybrid ratio when they are crossed.

However there has appeared a "unit-character" difference in one of these races of hooded rats. It appeared suddenly, and it Mendelizes when crossed with other

hooded rats. The occurrence of this "mutant" is claimed to have been induced by selection.

It seems to us quite improbable that this plus mutation could have arisen in the minus selection series. We believe that the repeated selection which was practiced had something to do with inducing this change in the plus direction (Castle and Phillips, :14, p. 31).

No reason for such a supposition is given. On the other hand there is clear reason for supposing that such a mutation would be far more easily detected in the plus series if it occurred there. The same mutation occurring in the minus race would perhaps have the same relation to that race as it had to the plus race when it occurred there; since it would lack the extension factors of the plus race, it would have a very different appearance and would probably have a grade not far from "0." It seems that very few rats of this grade were bred or tested. Had this mutation occurred in the minus race and been isolated, it would have been possible to obtain it as it appeared in the plus race, by proper crossing.

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SOME FEATURES OF ORNAMENTATION IN THE KILLIFISHES OR TOOTHED MINNOWS

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THE killifishes, so named by the early Dutch settlers about New York from their habit of living in the channels or kills, embrace an interesting family of fishes. They are known by other names, as top-minnows, cyprinodonts, toothed minnows, millions fish, etc. Some of these names are, however, more limited in scope and pertain to sections or genera. Top-minnow was applied from the habit of many living at the surface, and cyprinodont, meaning toothed carp, arose as some greatly resemble very small carps or true minnows (Cyprinidæ), though were found to differ in the presence of teeth in their jaws. Besides this character are a number of others, in which they agree with several related families to form the order of pike-like fishes (Haplomi). Such are all internal and largely have reference to the bony skeleton. In the abdominal ventral fins (*Procatopus* excepted), and without true spines in the dorsal and anal fins, the order resembles the herring-like fishes (Isospondyli), but differs in the absence of a mesacoracoid bone. This latter character is in agreement with the host of spiny-rayed fishes (Acanthopteri), but they usually have the ventral fins well anterior.

Though six families are included in the order of pike-like fishes, only the mud-minnows (Umbridæ) and the pikes (Esocidæ) occur in the Middle Atlantic States. The killifishes differ from both in the extremely protractile premaxillary bones, a condition very easily demonstrated by examining the upper jaw and prodding its edge forward. In form the body is oblong from elongate and slender to deep and nearly orbicular. The head is usually large and robust, often quite chunky. The mouth is small, with short gape, though wide and terminal. The teeth are extremely diverse, from broadly incisor-like to finely

villiform, and usually occur only in the jaws. The pharyngeal bones, unlike those of the true minnows or cyprinoids, often have fine teeth, rarely molar, and never modified or in even numbers as in cyprinoids. The scales are mostly large, cycloid, adherent, regular and without a perfected lateral line. The dorsal and anal fins are single, inserted usually behind the middle of the body, but no adipose fin developed. The caudal is broad and, though sometimes pointed, not forked. The paired fins are placed low, and the ventrals abdominal.

Many genera and species, about sixty belonging to the first and over three hundred to the last, have been described. Of these about ten genera and fifty species occur in the United States. The family reaches its greatest diversity in tropical America, and in the Old World the largest number of forms occur in African fresh waters. Killifishes live in fresh waters in nearly all situations, in lakes of great elevation, or in sandy desert streams, puddles and ponds. Others live in tidal waters, or along the shores of sea-beaches, and all near or close to the surface. The great changes with age, sex and season render many of the species difficult of determination. All are of small size, less than a foot in length.

In nearly all killifishes the sexual differences are well marked, at least during the spawning or breeding season. Often the males have enlarged fins, smaller in the females, as in the may-fish (*Fundulus majalis*) and the zebra-fish (*Fundulus zebrinus*). Still other characters occur in some species which have been entirely overlooked or scarcely noticed by most writers. These are the minute spines, or spinules, adorning the scales and fin-rays of certain species during the spawning-season. Garman, in his celebrated monograph of the killifishes,[1] simply says, "a minor sexual character is that of small spines appearing on the fins of males in several genera in the breeding time." I have been unable to find any detailed account of these structures, except casual reference to a few in descriptions of species. These are usually quite short and

[1] *Mem. Mus. Comp. Zool.*, XIX, 1895, p. 11.

of but slight value. So far as I have been able to examine material, these little spines occur only in certain species of the true killifishes, the pursy-minnows and the four-eyed fishes, or the Fundulinæ, Cyprinodontinæ and the Anablepinæ, respectively. I have never seen any in the top-minnows. It is interesting to note that the four-eyed fishes, creatures with remarkable and extreme modifications of structure, should be the only group of viviparous forms in which the spinules have so far been found to occur. These spinules are different in several ways from the nuptial tubercles of cyprinoids, in that they are more permanent, though very minute and inconspicuous. They may easily be overlooked in preserved examples, owing to the mucus exuded and covering the scales and fins. This should be carefully cleaned away, before they can be detected, and even then only with a good lens. Each spinule is found to arise on or close to the edge of the scale, and not on its exposed surface, as the more distinctly straight conic tubercles of the cyprinoids. The spinules are not always perfectly firm and rigid, but may be flexible or delicate. Those on the anal fin rays are generally curved slightly and are also often close together, though not perfectly regular. Their arrangement or design is usually more or less complete in each species. At least in one species their development occurs in the young, as in the ornatus stage of the common mummichog. Probably the spinules in most species are not permanent, but disappear after the spawning-season. However, if the spawning-season for a certain species is protracted, males with spinules may be found for a period of several months. Preserved specimens of killifishes do not show scars or pits like cyprinoids, and it may be that the spinules wear away as well as drop off. I have not found any examples with spinules in cold weather, or when spawning was apparently over. In no case have the inner edges of the pectoral rays been found with spinules, like the tubercles of certain cyprinoids. Doubtless such developments are to be correlated with the spawning habits, as none of the

EXPLANATION OF FIGURES

All the figures are drawn to the scale of millimeters and the accompanying numbers signify such, so that the number of times the line is contained in the lengthwise diameter of the figure will give its dimensions.

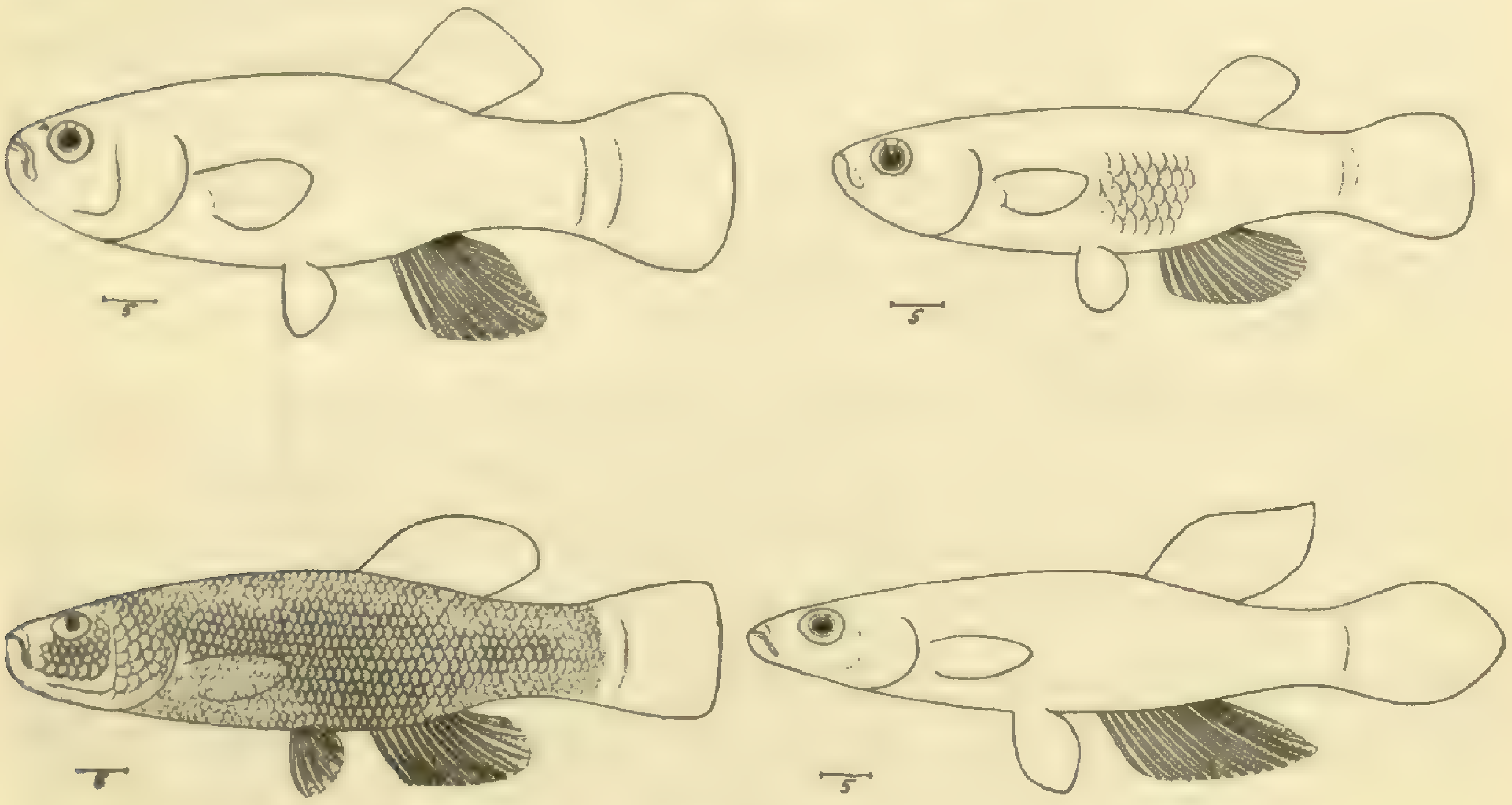

Plate 1

Fundulus nisorius Cope.
Fundulus zebrinus Jordan and Gilbert.
Fundulus floripinnis (Cope).
Fundulus stellifer (Jordan).

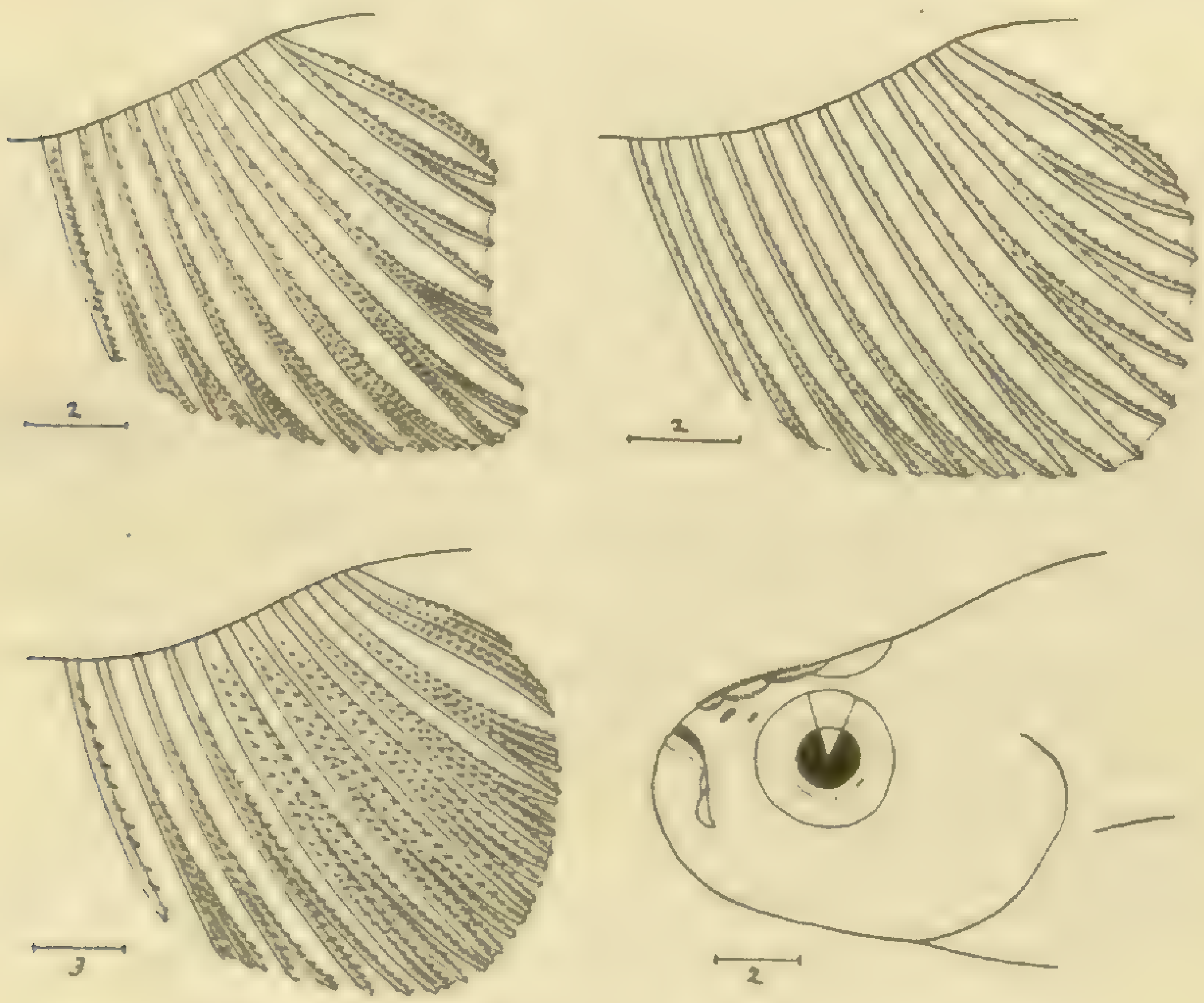

Plate 2

Fundulus heteroclitus macrolepidotus (Walbaum).
Cyprinodon bovinus Baird and Girard.
Fundulus diaphanus (Le Sueur).
Lucania parva (Baird).

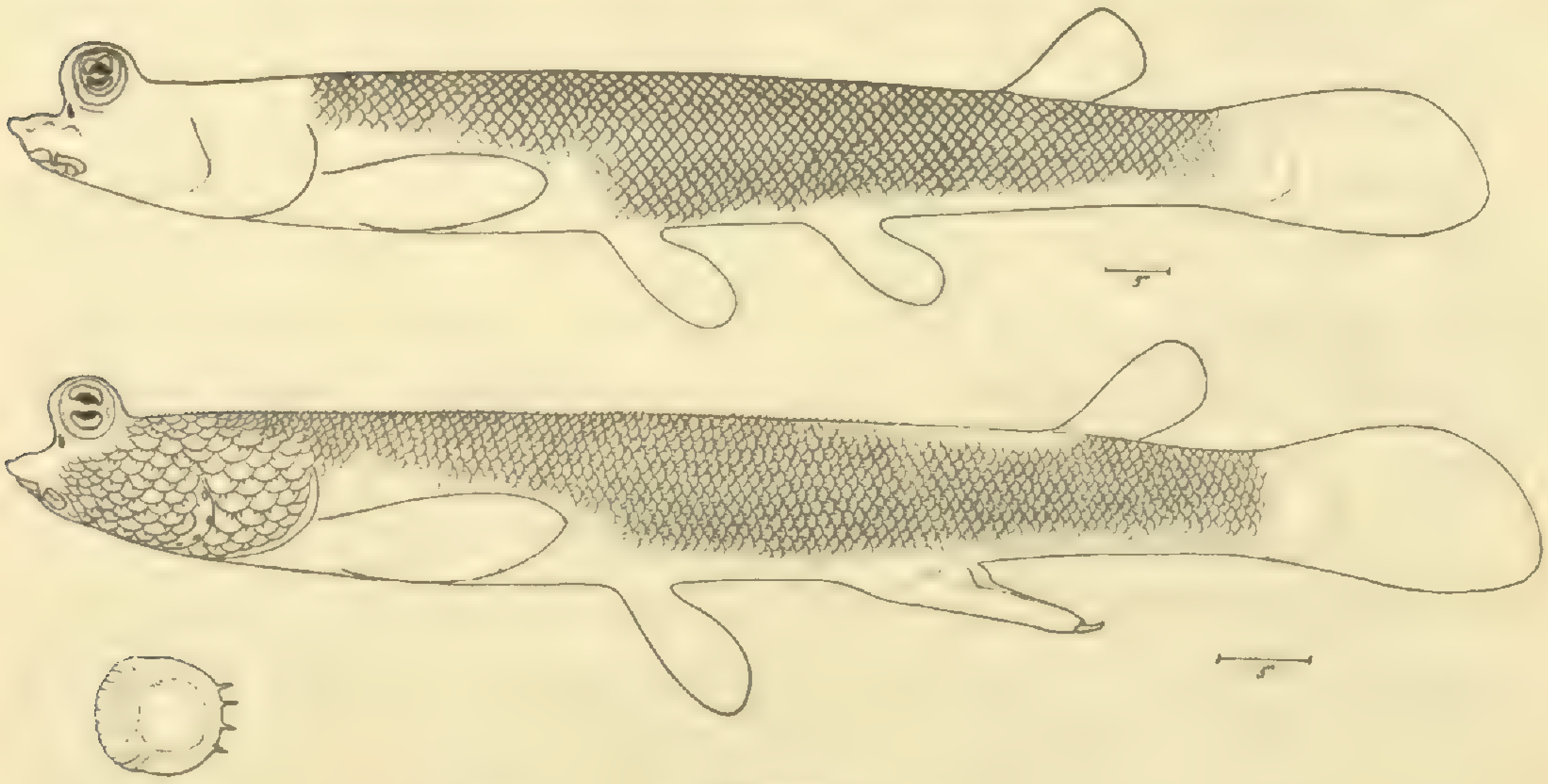

PLATE 3

Anableps anableps (Linné). Upper figure female, lower male, and enlarged scale to left.

killifishes have been seen to clasp the female as Reighard describes the creek chub (*Semotilus atromaculatus*). In the spawning behavior of the mummichog I could not determine if the male in any way secured or held fast to the female by means of his anal spinules, though possibly they may be of some such use. Killifishes greatly parasitized with sporozoa or myxosporidia have been found, the adult spawning-males sometimes greatly distorted, though with the development of the spinules more or less perfected. Among species of killifishes represented by spawning-males without spinescent ornamentation which I have examined are *Fundulus punctatus*, *F. similis*, *F. majalis*, *F. luciæ*, *F. nottii* and *F. notatus*.

In the common killifish or mummichog (*Fundulus heteroclitus macrolepidotus*) of the tidal waters of our Atlantic coast, the male is furnished with little spinules on the anal rays. They are better developed on the outer or terminal branches of the rays. They are also often irregularly placed, though usually a pair may be found on each segment, or as a spinule projecting out on each side of the fin. None of the scales or other fins with spinules. Spawning-males 76 to 82 mm. long. The female has a well-developed anal tube extending along the front of the anal fin for at least half the length of the depressed fin.

My examples 92 to 96 mm. Spawning fishes of this species were obtained from April until the middle of August.

In the West African killifish (*Fundulus nisorius*) the male has the outer portions of the anal rays covered with little spinules. It is also quite likely that the anal fin is furnished with spinules in the spawning *Fundulus bermudæ.*

The barred killifish (*Fundulus diaphanus*) common in the fresh waters of the east, from Maine to Carolina, is quite brilliant in the spawning-season. In the male the spinules are arranged as little points, like those of the mummichog, though as the fish is smaller they are less conspicuous. The scales and fins other than the anal are without spinules. Spawning males 60 to 70 mm. In the female a well-developed basal anal sheath extends around the front of the anal fin. Spawners of this species in full color were obtained from April until the middle of August.

The zebra-fish (*Fundulus zebrinus*) of the Mississippi Valley region has long been noted for its prickly appearance. Jordan and Evermann state, presumably with reference to spawning fish, "in males the margins of both dorsal and anal fins are evenly rounded, the anal the higher, its rays beset with minute white prickles." My examples show it differs from any of the preceding species in the male having the sides with the scales minutely spinescent along their edges. The area of spinescent scales extends from the head in some examples, in others for variable distances, back to caudal base, and always with its greatest development over the base of the anal fin. On the back the spinules gradually disappear, and the same is true on the under surface of the caudal peduncle. Further, an additional modification is seen in the presence of spinules on the inner or hind surfaces of the ventrals, though these fewer than on the anal rays. On the front of the anal fin the spinules are best developed, though irregularly distributed on the segments of the finrays, here and there appearing crowded or sparse.

Length 48 to 75 mm. The female has a broad basal sheath around the front of the anal fin. Length 51 to 63 mm.

The little green killifish (*Fundulus floripinnis*) of the South Platte River basin has the male with the scales along the middle of the side, especially above the base of the anal, with minute prickles along their edges. Similar prickles also occur on the rays of the anal fin, though with irregular distribution on the segments. They usually appear better developed along the front anal edge. I have also seen a few minute prickles above the eyes. In length these males were 47 to 57 mm. This species belongs to the section *Zygonectes* Agassiz, so called as the fishes were said to swim in pairs. Doubtless this would refer to the spawning-habits or when spawning, for at other times they do not appear to swim in pairs. As in the brown killifish (*Fundulus luciæ*), another member of the *Zygonectes* group, I have never seen them swimming in pairs, and Ellis claims the same for the little green killifish.

In the stud-fish (*Fundulus stellifer*) the males have very minute spinules along the anal rays and along the edges of their scales above the fin. They are also irregularly placed. Length 82 to 99 mm., and the females 73 mm. long have a well-developed basal anal sheath at the front of the fin. The related *Fundulus catenatus* shows similar ornamentation in the male, though my material is inadequate for detailed comparison.

In the rainwater-fish (*Lucania parva*) males in high color, taken in June, differ from any other killifish I have examined in the presence of minute spinules on the upper surface of the snout, in some cases even encroaching on the interorbital space. No other spinules occur. The muzzle of the male is also modified or decidedly obtuse, suggestive of the fat-head minnows (Pimephales).

The pursy-minnows (*Cyprinodon variegatus*) when in brilliant spawning-dress, in the case of the males, are extensively provided with minute spinules. These extend all along the edges of the scales on the head, front predor-

sal region, posterior sides of trunk or above anal fin, and front side of caudal peduncle. All the anal rays are also minutely and finely spinescent, though I have not found any spinules on the paired fins. Spawning-males 54 to 57 mm., and the females smaller. The related *Cyprinodon bovinus* of the southwest is similar. *Jordanella floridæ* is represented only by one small example with spinules, these very minute along the edges of the scales above the anal. No spinules found on any of its fins.

In the four-eyed fish (*Anableps anableps*) of South America, the males have an intromittent organ dextral or sinistral. They also have the scales on the trunk, especially above the anal and on the predorsal region, with spinules, though more numerous or dense with spinules in the former space. Top of head, belly and lower surface smooth. Sides of caudal peduncle with a few scattered spinules. A large female, 244 mm. long, is largely spinescent on the trunk above, though the spinules not so dense as on scales above the anal in the male. In females of smaller size, 124 to 128 mm. long, the spinules are rather obsolete, sparse and scattered, also only on the back and sides above. Young 27 mm. long still show the umbilical sac well developed and are scaleless.

Though I have not examined spawning examples of small-finned killifish (*Fundulus parvipinnis*), Jordan and Gilbert state, "scales large; in the males in spring roughened or ctenoid by small granulations and prickles, similar to the nuptial excrescences of some Cyprinidæ; fins also rough."

SHORTER ARTICLES AND DISCUSSION

FURTHER REMARKS ON THE INHERITANCE OF CONGENITAL CATARACT

An article[1] by the writers in a previous number of this publication dealing with the inheritance of congenital cataract in a statistical way has been rather severely arraigned by Danforth[2] in a more recent issue. In our original paper we presented data taken from genealogical tables published by Harman in the "Treasury of Human Inheritance" which led us to believe—

1. That congenital cataract could no longer be considered as a single, dominant, unit character.

2. That Davenport should be criticized for making eugenical recommendations based on the inheritance of cataract as a dominant character when the method of inheritance is not positively known.

3. That from the evidence at hand cataract could better be considered as a single, recessive, unit character, reserving final decision as to this point until more complete data should become available.

Danforth believes with us that congenital cataract can not be considered as a simple, dominant character. Nevertheless he tries to defend Davenport from "unjust" criticism while he does not agree with him. It is upon the assumption of cataract as a positive or dominant character that Davenport bases his eugenical recommendation as follows:

> The usual method of inheritance is that of a positive character. Affected individuals have either half or all of their offspring affected, while two unaffected parents will probably not have defective offspring. However, as cataract usually appears late in life it is not always possible to predict whether the parent will become affected or not.
>
> The eugenic rule is this: If either parent has cataract at least half of the offspring will have it also. If a person belongs to a strain that has cataract but is free from it, advice must depend on the nature of the cataract. If in the family strain cataract appears early, before the age

[1] Jones, D. F., and Mason, S. L., "Inheritance of Congenital Cataract," The American Naturalist, 50: 119–126, February, 1916.

[2] Danforth, C. H., "Inheritance of Congenital Cataract," The American Naturalist, 50: 442–448, July, 1916.

of the person who contemplates marriage, then such marriage may be advised; . . .[3]

As regards congenital cataract, then, Davenport advises that unaffected persons from affected stock can marry without fear of producing affected children. Harman's tables show over thirty matings of unaffected parents having at least one affected child.

No matter how unsatisfactory is the proof that cataract is a simple recessive, it should be borne in mind that the data given in Harman's tables do not stand the test when cataract is considered as a simple, dominant character. If the argument that heterozygous individuals sometimes show the recessive character is to be used to prove the dominance of cataract, it would be necessary to use the assumption to explain thirty-one exceptional families which have from one to eleven children of which 40 per cent. of the total are affected. On the recessive hypothesis there is only one exceptional family so far known to be explained. As long as it is not a simple dominant character it makes no difference whether it is a simple or complex recessive or a dominant governed by multiple factors, the eugenical recommendation quoted above should not be made, and we still believe that Davenport can be justly criticized.

Danforth objects to the disagreement between the observed and the expected results in our table I, giving the progenies of matings of normal by normal, and compares the goodness of fit unfavorably with data given by Usher on retinitis pigmentosa. In our results the disagreement lies in an excess of the actual number of affected children over the expected number. If, as Danforth says, "a certain number of congenital cataracts are produced by intrauterine poisoning without necessarily any reference to heredity" the tendency would be to raise the actual number of affected children above the expected. Also any cases of *origin de novo,* to which he believes we did not give enough consideration, would tend to have the same effect. Moreover, it should be noticed that Usher has over twice as many individuals to base his ratio upon, 320 as compared to 153 in our case.

Danforth states two main conditions which he says our assumption of cataract as a recessive character does not meet. The first is the low probability of an individual carrying the abnor-

[3] Davenport, C. B., "Heredity in Relation to Eugenics." Henry Holt and Co., New York, 1911, pp. 111–112.

mality in a haploid or a diploid state meeting with a heterozygous normal, in random mating, which would be necessary to produce affected children. According to Danforth's calculations the probability of heterozygotes in the general population is one in thirty. He shows that Harman's tables give in some cases as high as eight out of nine individuals mating with normals and producing affected children, thereby showing that the normals are heterozygous on the recessive hypothesis. In the previous publication we did not give consideration to this point which is of noteworthy significance and we are indebted to the writer for calling our attention to it.

This apparently high proportion of heterozygotes in the general population would be a serious objection to our simple recessive hypothesis if it were not for the fact that there is a considerable amount of consanguinity recorded in the pedigrees given by Harman. With each of the pedigrees including from one to many families there is a definite statement as to whether a record was made and, if so, whether or not consanguinity was present. Tabulating these statements shows that in sixty of the pedigrees no record was made. In twenty-four no consanguinity and in eleven consanguinity was definitely recorded. Then in those cases in which a record was made nearly 50 per cent. of the pedigrees show more or less intermarrying. Altogether there are seventeen cousin marriages.

With this amount of intermarrying among affected stocks the proportion of heterozygous individuals carrying the abnormality in a simplex condition would be greatly increased over the proportion in the general population, and Danforth's most serious objection to our hypothesis loses its force. Evidently no consideration was given to this point when he says "a more striking refutation of the assumption could hardly be found" (p. 447).

With regard to the second main condition which is raised against the assumption of cataract as a single, recessive, unit character Danforth seems to be partly in error, if we understand his statement correctly. He states: "If congenital cataract were recessive the normal children of a cataractous parent should themselves produce affected children in half as many cases as do their cataractous sibs, and the total number of affected children produced should be one half as great in the first case as in the second" (p. 446). Since on the recessive hypothesis only heterozygous normal and homozygous abnormal sibs are produced

in equal numbers from matings of Nn $\times$ nn included in category B, and neither can produce affected children in turn, unless mated to a heterozygous normal or a recessive, we do not see why the normals should produce affected children in *half as many cases* as their cataractous sibs. If the chances for obtaining such mates were the same the number of matings which produce affected children should be approximately the same.[4]

The second part of the quotation is correct only when both normal and abnormal F_1 individuals have an equal chance to mate with individuals who are either affected or carry the abnormality in a recessive condition. The chances of the two classes mating with such individuals are probably not equal because individuals affected with cataract would have a harder time to find a mate than their normal brothers or sisters and there would be a greater tendency towards consanguineous marriages and consequently a greater chance of mating with cataractous individuals. The frequent intermarrying among affected stocks is well known with other abnormalities. Hence normal persons carrying the affectation in a heterozygous condition would be more likely to marry into unrelated stocks with a far less proportion of heterozygous individuals than would their affected sibs. If this is true then the expectation of the number of matings of affected F_1 individuals giving affected children in turn

[4] In answer to a letter sent to Dr. Danforth asking about the above point the following was received which shows that we did not understand his meaning correctly:

In reply to your letter of September 29, I do not say in the paragraph to which you refer that of the children of cataractous parents half as many normal as cataractous *individuals* should produce affected offspring but, on the contrary, that the normals, taken as a group, should produce affected children "in half as many cases" (*i. e., at half as many births*) as do the cataractous. The families of *numerous individuals* in both groups would be expected to contain no cataractous individuals at all, but in those families (equal number for each group) where such children may occur there should be in the long run half as many cases in families with normal parents as in families with one cataractous parent. The remainder of the paragraph to which you refer and the statements in your letter show, I think, that we are in complete agreement as to the theoretical expectations; it was my unfortunate use of the word "cases" which no doubt caused you to raise the question. I meant it to refer to F_3, you doubtless suspected it might refer to F_2. This suspicion was undoubtedly strengthened by my "relation of one to two" which is one of those slips of the pen for which I have no means of accounting. Of course it should have been "one to one" and that fact was uppermost in my mind at the time of writing the passage!

would be greater than that of the unaffected and the total number of children would therefore be more than twice as great.

Danforth, however, after raising this condition does not determine the number of affected children from the affected and unaffected F_1 individuals, but calculates the percentages of these two classes of parents which produce at least one affected child. He finds that eighty-six per cent. of the cataractous children of a cataractous parent themselves produce some affected children and thus presumably have mated with heterozygous normals. Of the normal children from the same F_1 generation only ten per cent. produce affected children. If the chances for securing similar mates were the same these percentages should be approximately equal. The relation of ten to eighty-six which does not conform to a one to two ratio as Danforth states that it should necessarily deviates still more widely from a one to one ratio.

There are two reasons why this deviation from a one to one ratio can be expected in favor of a larger number of affected matings giving affected children than of unaffected matings. The first lies in the fact that matings of affected by heterozygous normals should give a one to one ratio of affected and normal children, whereas the matings of heterozygote by heterozygote should give a ratio of one to three. As was emphasized in our previous publication the only criterion by which it can be determined whether the mates to the two kinds of F_1 individuals are heterozygotes or homozygotes is the production of at least one affected child. In families with a small number of children the matings which promise a one to one ratio would have a greater chance of producing at least one affected child than matings which promise a ratio of one affected to three unaffected children. Hence more of the families of the latter than of the former class would be omitted from the data.

The other reason why the deviation that Danforth obtains can be expected is that which has already been mentioned, namely, that affected individuals are more likely to marry related individuals because of the greater difficulty of obtaining a mate than the unaffected would have. The proportion of heterozygotes in affected strains would be far higher than in the general population, so that the chances of the two kinds of F_1 individuals mating with a heterozygous normal would not be equal as Danforth considers them to be.

It is recognized that these arguments are extremely indefinite and that it is difficult to determine just how much value to give

them. They are however hardly necessary since the numbers ninety-six and forty-seven upon which Danforth bases his criticism are too small to make a really critical comparison.

Since the number of affected F_1 individuals which should give one half affected children exceeds the number of unaffected F_1 individuals which should have only one third affected children, the actual number of affected children in the two kinds of F_2 populations would deviate proportionally farther from a ratio of one to two. If it is conceded that the chances for the two kinds of matings are not equal, then this deviation would be expected.

The three cases in category C which we gave as matings of abnormal by abnormal which theoretically should give only abnormal children according to the simple recessive hypothesis can be found in Harman's tables in the "Treasury of Human Inheritance"[5] as follows: Table 309, Parents I, 1 and 2—Children II, 1 to 5; Table 312, Parents II, 3 and 5—Children III, 3 to 4; and Table 342, Parents III, 28 and 37—Children IV, 60 to 66. Danforth says that he can find only two of these. They are probably 309 and 342. The one which occurs in Table 312 should not have been used without an explanation. Although the chart indicates that both parents are affected as well as their two children, the description of the family shows that the exact parentage is somewhat in doubt. It was an error on our part not to mention this fact.

With regard to the family in 342 in which part of the children are normal where only abnormals are expected, Danforth does not accept our explanation that heterozygotes sometimes have the recessive character. This is quite frequently shown in other material. His refusal to accept this explanation to account for the one exception to the recessive hypothesis is shown in the following quotation: "a single bona fide case in which two affected individuals produce normal offspring is sufficient to overthrow it" (the recessive hypothesis) (p. 447). We can not understand his refusal to accept this explanation to account for one exception when he is willing to use it to explain thirty-one exceptions to the dominant hypothesis! This is evident from the following quotation previously alluded to:

Again, since Jones and Mason elsewhere in the same paper (p. 124)

[5] Harman, N. B., "Treasury of Human Inheritance," Eugenic Laboratory Memoirs, XI, Part 4, Section XIIIa, pp. 126–169, Pl. XXVIII–XXXIII, Dulau and Co., London, 1910.

use the same argument that "heterozygous individuals sometimes show the recessive character," we might, if necessary, use the same argument to prove the dominance of cataract. On the assumption that congenital cataract is dominant instead of recessive it might be maintained that in those cases where both parents of affected individuals seem to be normal, one of them is, after all, heterozygous—and affected children are therefore to be expected (p. 444).

Perhaps Danforth would be willing to consider another explanation which he suggests, that somatic cataracts of a congenital origin are not uncommon. If one of the parents in question had a somatic cataract the appearance of normal children would be expected but not of affected children unless the parent was also heterozygous for hereditary cataract. A probability which would be rather remote but not impossible.

From the data as they have been gathered up to this time it seems impossible to arrive at an explanation of the mode of inheritance of cataract which will be entirely satisfactory. While more proof is awaited, we believe that the assumption of congenital cataract as a single, recessive, unit character has the best support from the facts at hand. The article by Danforth has brought out several important considerations which we neglected. It is regretted that in this paper which at first sight makes out a strong case against our recessive hypothesis there is nothing offered towards a different solution of the problem.

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THE STATUS OF FOWLER'S TOAD, *BUFO FOWLERI* PUTNAM

S. P. Fowler, of Danvers, Essex County, Massachusetts, appears to have been the first to recognize the fact that this toad differed in many respects from the common toad. In a letter[1] to Prof. F. W. Putnam, Fowler gave a very accurate and complete account of the song and habits of this toad as he had observed it around Danvers.

Cope (see loc. cit.) discussed in much detail *Bufo lentiginosus fowleri* (Putnam). Little was known of this toad at the time Cope wrote. In fact, Cope stated that it was confined to a few

[1] Cope, E. D., "The Batrachia of North America," Bull. 34, U. S. National Museum, 1889, pp. 279–281.

ponds in northeastern Massachusetts, near the town of Danvers. He says:

Such a limited distribution for a land vertebrate is remarkable, as is also the fact of its having so long remained without introduction to science.

Cope's work was published in 1889, in the same year that Allen[2] reports having heard Fowler's toad in New Hampshire. Speaking of *Bufo americanus* Le Conte, Allen said:

After the breeding season, the toad's song changes from a prolonged pipe to a shorter, lower-toned note, that, at night, has a peculiar weirdness, and almost reaches a wail.

Although Allen thought that the common toad was responsible for the two songs, it is plain that he had heard the unmistakable song of Fowler's toad. Allen's observation extended the range of this toad well up into New Hampshire.

Although as late as 1889 Fowler's toad appeared to have a very local distribution in New England, more recent work has shown that this toad has an extended range southward.

In 1907 the writer[3] published a paper showing that Fowler's toad is very common around Oxford and Worcester, in Worcester County, Massachusetts. In a second paper, published in 1908,[4] it was shown that the range of this toad extended through Washington, D. C., and Chapel Hill, North Carolina, into northern Georgia, where it appeared to be the only common form in the vicinity of Hoschton and Thompson's Mills, near Gainesville.

In 1910 Miller and Chapin[5] gave an excellent discussion of the range of *Bufo americanus* and *Bufo fowleri* in New Jersey and adjacent regions of New York.

From the observations of Miller and Chapin it appears that Fowler's toad occupies practically the entire state of New Jersey, except, perhaps, the extreme northwestern part. Throughout

[2] Allen, Glover M., "Notes on the Reptiles and Amphibians of Intervale, New Hampshire," *Proc. of the Boston Society of Nat. History*, Vol. 29, No. 3, 1889, p. 71.

[3] Allard, H. A., "Fowler's Toad, *Bufo fowleri* Putnam," *Science*, N. S., Vol. 26, No. 664, Sept. 20, 1907, pp. 383–384.

[4] Allard, H. A., "*Bufo fowleri* in Northern Georgia," *Science*, N. S., Vol. 28, No. 723, Nov. 6, 1908, pp. 655–656.

[5] Miller, W. De W., and Chapin, James, "The Toads of the Northeastern United States," *Science*, N. S., Vol. 32, No. 818, Sept. 2, 1910, pp. 315–317.

central and southern New Jersey it is the only species, as *B. americanus* was not found here. Miller and Chapin also found that Fowler's toad was the only form to be found upon Staten Island, N. Y., as well as upon Long Island. In the mountainous parts of northern New Jersey both *B. americanus* and *B. fowleri* occur.

In 1914 Overton[6] published an interesting paper concerning the frogs and toads of Long Island. Overton found that *Bufo fowleri* is the only toad occurring on Long Island, where it appears to be common, while the common toad of the mainland of New York State is *B. americanus.*

Various authors have mentioned the song of *Bufo fowleri.* S. P. Fowler in the letter to Professor S. W. Putnam, previously cited, first described its song. His description is particularly apt.

> To my ears the croak is a sharp, disagreeable, unearthly screech, difficult to describe, as it is unlike any sound I have ever heard. A chorus of these has been likened to the whoop of a party of Indians.

As none of us at this late day can recall the whoop of Indians, this comparison, although historically interesting, does not give us much aid in appreciating the peculiar nature of the sound.

Dr. Nichols, in the same letter, is cited as considering the song to be a shrill monotone in a high falsetto voice, longer and more trilling than the voice of Pickering's *hyla.* Fowler, however, states that there is no trill to the note, an opinion the writer also shares.

The writer has described the note as follows: "I have heard nothing in nature so weird and unearthly as the almost agonized wail of this toad, repeated at intervals,"[7] and "The usual note of Fowler's toad is a brief, penetrating, droning scream."[8]

Miller and Chapin, in their article previously cited, say of it:

> . . . it certainly has much less music to it than the trill of the American toad. The notes are more closely connected, so that a sort of buzzing is heard.

Miss Dickerson[9] says of the notes of *Bufo fowleri:*

6 Overton, Frank, "The Frogs and Toads," Long Island Fauna and Flora, III. In the Museum of the Brooklyn Institute of Arts and Sciences, *Science Bulletin*, Vol. 2, No. 3, Nov. 3, 1914.

7 *Science*, N. S., Vol. 26, No. 664, Sept. 20, 1907.

8 *Science*, N. S., Vol. 28, No. 723, Nov. 6, 1908.

9 Dickerson, Mary C., "The Frog Book," 1906.

The call of the Fowler's toad is a metallic, droning sound, not conspicuously vibrated. The pitch of the call may be as high as that of *Bufo americanus,* but descends in doleful fashion through several intervals before the close. Its carrying power is unusually great. The quality is indescribable; on the whole, the call is weird and mournful and not especially agreeable to our ears.

Overton (previously cited) says:

Its song is a combination of a low whistle and a moan, and the two sounds do not melt into a chord. The combined sound is discordant and decidedly unpleasant to a musical ear, but at a distance the sound is more pleasant for the moan is not apparent and only the whistle is heard. The sound lasts from two to three seconds and may be repeated at intervals of about ten seconds.

Overton says the song of *Bufo americanus* is prolonged about thirty seconds.

Dr. Andrew Nichols,[10] of Danvers, Massachusetts, is quoted as saying:

There is no sound in bog, pond, fen, forest, or air at all like it.

Although Nichols referred to the toad as *Bufo lentiginosus* Shaw, it is extremely probable that he had in mind *Bufo fowleri.*

Miss Hinckley[11] says:

The bleat of *B. fowleri,* with its far reaching, metallic ring, is usually heard after sunset. I have seen the latter give voice on the land, while the trill of *B. americanus,* heard at all times of day and night during the mating season, I have only seen given in the water.

In the field the writer has found little difficulty in recognizing Fowler's toad throughout its range. Its note at once distinguishes it from *B. americanus.* Color characters, while fairly definite, do not, perhaps, always serve to distinguish *B. fowleri* from *B. americanus.* According to Miller and Chapin, the color of the eye alone will distinguish *B. fowleri* from *B. americanus.* These observers state that in the former the iris is silvery, while in the latter it is bronze. There is some question in the writer's mind as to the value of this character as an identification mark. The question is now under investigation.

10 Nichols, Andrew, *Proc. of the Boston Soc. of Nat. History,* Vol. 1, Aug. 2, 1843, p. 136.

11 Hinckley, Mary C., "On Some Differences in the Mouth Structure of Tadpoles of the Anourous Batrachians Found in Milton, Mass.," *Proc. of the Boston Soc. of Nat. Hist.,* Vol. 21, 1882, pp. 307–314.

Miss Dickerson states that the eggs of *Bufo fowleri* are often arranged in double rows, but that, so far as known, the eggs of *B. americanus* are always laid in single strings. If these characteristics hold true for the two toads it would appear that the toad with which Gage[12] worked was *Bufo fowleri,* rather than *Bufo lentiginosus americanus.* Speaking of the toads with which he worked, Gage states that they lay their eggs from the middle of April until the middle of June, and that the eggs were laid in two strings, one from each oviduct. The lateness of the egg-laying season adds to the probability that Gage worked with *B. fowleri* rather than with *B. americanus.*

From the observations of various observers, it is evident that *Bufo fowleri* is a widely distributed toad and is extremely abundant in many places from New Hampshire, throughout New Jersey, the District of Columbia, southward at least as far as Gwinnett, Jackson and Hall Counties in northern Georgia. Cope (previously cited) records a specimen of this toad from New Harmony, Posey County, Indiana. He also states that a specimen of the variety *B. lentiginosus* var. *americanus* from Nebraska approximates so nearly *B. fowleri,* that the latter can not be regarded as under all circumstances separate and specific in its rank.

Miller and Chapin have found that toads taken on the Palisades and on the northern end of Manhattan Island sometimes show forms intermediate between *B. americanus* and *B. fowleri.* These observers have suggested that such intermediate forms may represent hybrids, but, as they state, it is a question for experimental study.

For a long time the writer has had in mind the question of experimental hybridization between typical forms of *B. fowleri* and *B. americanus.* It would be of considerable interest to determine whether or not these two toads can be hybridized. Although *B. fowleri* is more sensitive to lower temperatures than *B. americanus,* and lays its eggs later in the season, it should not be especially difficult to provide conditions that would bring the mating season of the two toads together under temperature conditions required by *B. fowleri.* It is very probable that the hibernation period of *B. americanus* could be prolonged by artificial refrigeration until the mating and egg-laying period of *B.*

[12] Gage, S. H., "Hibernation, Transformation and Growth of the Common Toad (*Bufo lentiginosus americanus*)," Ithaca, N. Y., *Proc. of Amer. Assoc. for the Advancement of Science,* 47: 1898.

fowleri had arrived. If experimental hybrids could be obtained, it would be especially interesting to compare the voices of the hybrids with the voices of the parents, as well as to determine the hereditary behavior of various other characters.

In those localities where both toads are found, differences in behavior peculiar to each species tend to prevent natural cross mating. *Bufo americanus* is the first toad to appear and, at least around Oxford, Massachusetts, has completed egg-laying and left the water long before *B. fowleri* has appeared. Furthermore, the preference that *B. fowleri* shows for certain ponds from year to year is rather remarkable.

Fowler (letter previously cited) noted that only certain ponds around Danvers, Massachusetts, were visited by *B. fowleri*. In the region of the writer's early home, Oxford, Massachusetts, the same rigid preference was shown for certain bodies of water during the mating season. Here it was indicated that these toads traveled very long distances to reach a certain quiet bend in the Maanixit River. Although other permanent bodies of water were near, these, for some reason, were never visited by these toads.

The writer hopes that an interest in our common toads will finally lead some one to investigate the possibility of experimental hybridization between *B. americanus* and *B. fowleri*, and the question of the relationship of these toads. Batrachian hybridization seems never to have been undertaken. It would appear that such investigations would throw much light on the question of geographic variation, intergrading forms, etc. Few creatures are more companionable and harmless in their behavior and more useful to the agriculturist as insect destroyers, than the toads. Knowledge of their habits, relationship, etc., is not only of scientific, but also of soundly practical interest.

ADDITIONAL REFERENCES IN THE LITERATURE TO FOWLER'S TOAD

Holbrook, J. E. *North American Herpetology*, Vol. 5, 1842. Speaking of *Bufo lentiginosus* Shaw, he says the males seek the females in the month of May when hundreds may be seen together in some stagnant pool depositing their eggs. Of the notes he says: "The males at this season are extremely noisy, though at other times they are silent, or make only a slight chirp when taken" (p. 9).

Gorman, Samuel. The North American Reptiles and Batrachians. *Bull. Essex Inst.*, Vol. 16, 1884. On page 42 he says of *B. fowleri* Putnam: "This is an *americanus* of moderate size and with frontal ridges low,

close together, and nearly or quite parallel. Voice peculiar. Manitoba to Winnipeg; Massachusetts."

Cope, E. D. Check List of North American Batrachia and Reptilia with a Systematic List of the Higher Groups and an Essay on Geographical Distribution based on the Specimens contained in the U. S. Nat. Museum. 1875. P. 29, *B. lentiginosus,* subspecies *fowleri* is given a distribution from Massachusetts to Lake Winnipeg. Lists *B. l. fowleri* (page 86) as confined mostly to the Canadian District of the Eastern Region.

Hay, O. P. The Batrachians and Reptiles of Indiana, 17th Ann. Rept. of Dept. of Geol. and Nat. Resources of Indiana, 1891. *B. fowleri* considered a variety of *B. lentiginosus.* Range given as Danvers, Mass., and the fact that Cope reported a specimen from New Harmony, Indiana (p. 459).

Sherwood, W. L. The Frogs and Toads Found in the Vicinity of New York City. Abst. No. 10 of the Proc. of the Linn. Soc. of N. Y. for year ending 1898. Mentions *B. fowleri* as a subspecies of the common toad, stating that it was confined to northeastern Massachusetts.

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Ditmars, Raymond L. The Batrachians of the Vicinity of New York City. *The American Museum Journal,* Vol. 5, 1905. Speaking of the common toad, he says there are four varieties, one of which occurs only in northeastern Massachusetts.

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Hancock, J. L. The Toad's Social Life in Nature. Sketches in Temperate America, 1911. Fowler's toad is briefly mentioned and illustrations are shown.

Surface, H. A. First Report on the Economic Features of the Amphibians of Pennsylvania. Zoological Bulletin of the Div. of Zoology, Pennsylvania Dept. of Agriculture, Vol. III, Nos. 3 and 4, May–July, 1913. On page 114, *B. fowleri* is discussed. Statement made that it has been recorded from New England and New York.

H. A. Allard

Washington, D. C.,
May, 1916

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